Functional Analysis of the Architecture of the Oro-facial and Hyoid Musculature: A Comparative 3D Modelling Study.

by

Zhi Li

A thesis submitted in conformity with the requirements for the degree of Master of Science
Institute of Medical Science
University of Toronto

© Copyright by Zhi Li 2015
Functional Analysis of the Architecture of the Oro-facial and Hyoid Musculature: A Comparative 3D Modelling Study.

Zhi Li
Master of Science
Institute of Medical Sciences
University of Toronto
2015

Abstract

The functions of the oro-facial and hyoid muscles are poorly understood, despite their importance in mastication and communication. Muscle architecture is an important determinant of function, but the lack of an architectural database is impeding understanding of the individual and coordinated activities of these muscles. The purpose was to digitize, model and analyze the architecture of the oro-facial and hyoid muscles to compare their force-generating and excursion capabilities, as determined by the muscle architectural parameters, at the muscle group, whole muscle, and muscle partition levels. In total, 9600 fibre bundles were digitized and modelled in 46 muscles. The functional characteristics of each muscle and its component parts were determined from the architectural parameters, line(s) of action, and force indices. Correlation of the volumetric musculotendinous data with the functional characteristics of a muscle provides a comprehensive approach to assessing the implications of muscle geometry in normal and pathological states.
Acknowledgments

In completing my research project and writing my thesis, I benefited greatly from the expertise, guidance, and kindness of many people. Foremost, I owe my sincerest gratitude to my supervisors Dr. Anne Agur and Dr. Nancy McKee for providing me with an amazing opportunity for me to grow and mature as a scientist and as a well-rounded individual. To Dr. Anne Agur, I am deeply indebted in your kindness, dedication and guidance. I am very fortunate to have you as my mentor, someone who always found the time to listen and provide advice on problems that arose during my academic and personal journey over the last four years. To Dr. Nancy McKee, thank you for all of the thought-provoking discussions and for sharing your clinical experiences with me which allowed me to see the importance of my research.

Many thanks to my Program Advisory Committee members Dr. Karl Zabjek and Dr. Denyse Richardson for their guidance, encouragement and assistance every step along the way, from the development of ideas to the final editing of my thesis. I also thank my Examination Committee members Dr Cathy Amara, Dr Scott Lozenoff and Dr Paulo Koeberle for their stimulating and thoughtful questions and comments.

My sincere appreciation also goes out to my fellow graduate students. Special thanks to Shannon Roberts for meticulously and painstakingly reading over the many drafts of my thesis. Your help and editorial advice was invaluable in elevating the quality of this dissertation. To Dongwoon Lee, thank you for all the timely assistance in helping me troubleshoot the various obstacles in the data analysis process. I also greatly appreciate your guidance in developing my understanding of geometric analysis and computer sciences concepts. To Kate Sauks, thank you for all of your support, advice and your uplifting spirit.
To the members of Parametric Human Project Azam Khan, Dr. Sid Fels, Jeremy Mogk, Jacky Bibliowicz, Antonio Sanchez, Dr. Alan Hannam and many others. Thank you for enabling me to see how my research fit into the grand picture, and for providing me with a supportive and stimulating research environment.

To my parents, who showed me the importance of courage, hard work and persistence, I owe my deepest gratitude for all the sacrifices you made and the life you have created for me. To my sister, “yes, brother has finished grade eighteen!”

Last but not least, to my wonderful partner Marianne Pasiliao. Thank you for all your tireless support, and unyielding love. I am very lucky to have you accompanying me on my journey.

Acknowledgement is also made to Ontario Graduate Scholarship Program, Natural Science and Engineering Research Council Canada Graduate Scholarship for their support, and Autodesk Inc. for providing research license for Autodesk® Maya® 2013 (Autodesk Inc. San Rafael, CA, USA:http://www.autodesk.com/maya).
Table of Contents

Acknowledgments ................................................................................................................... iii

Table of Contents ................................................................................................................... v

List of Abbreviations ................................................................................................................. ix

List of Tables ........................................................................................................................... x

List of Figures .......................................................................................................................... xii

Chapter 1 .................................................................................................................................... 1

1 Introduction ............................................................................................................................ 1

1.1 Contents of thesis ............................................................................................................... 2

Chapter 2 .................................................................................................................................... 3

2 Literature review ................................................................................................................... 3

2.1 Introduction ........................................................................................................................ 3

2.2 Contractile elements ......................................................................................................... 3

2.2.1 Macroscopic structure ................................................................................................. 3

2.2.2 Microscopic structure .................................................................................................. 4

2.2.3 Contractile mechanism ............................................................................................... 5

2.2.4 Length-tension relationship ....................................................................................... 8

2.3 Connective tissue elements ............................................................................................. 11

2.3.1 Macroscopic structure ............................................................................................... 11

2.3.2 Microscopic structure ............................................................................................... 13

2.4 Muscle Architecture ......................................................................................................... 14

2.4.1 Fibre bundle length .................................................................................................... 16

2.4.2 Pennation angle ......................................................................................................... 16

2.4.3 Muscle volume .......................................................................................................... 18

2.4.4 Physiological cross-sectional area and force index .................................................... 19
2.4.5 Sarcomere length ................................................................. 19
2.4.6 Tendon geometry ................................................................. 20
2.5 Measurement of architectural parameters ...................................... 20
  2.5.1 Two-dimensional measurements .............................................. 21
  2.5.2 Three-dimensional measurement ............................................. 24
2.6 Architecture of the oro-facial and hyoid muscles ................................ 25
  2.6.1 Cadaveric studies ................................................................. 25
  2.6.2 Imaging studies ................................................................. 31
2.7 Muscle modelling ................................................................. 34
  2.7.1 Line segment models .......................................................... 34
  2.7.2 Volumetric muscle models ................................................... 35
2.8 Summary ............................................................................. 36
Chapter 3 ............................................................................... 38
3 Objectives and hypothesis .......................................................... 38
  3.1 Objectives ........................................................................ 38
  3.2 Hypothesis ....................................................................... 39
Chapter 4 ............................................................................... 40
4 Material and Methods ............................................................... 40
  4.1 Scanning of specimen ............................................................ 41
  4.2 Dissection and digitization ....................................................... 42
    4.2.1 Digitization of fibre bundles .............................................. 42
    4.2.2 Digitization of tendinous elements .................................... 43
  4.3 Digitization of oro-facial and hyoid musculature ......................... 44
    4.3.1 Muscles of facial expression .............................................. 44
    4.3.2 Muscles of mastication .................................................... 45
    4.3.3 Supra- and infrahyoid muscles ......................................... 45
## 4.4 Three-dimensional modelling of digitized data .................................................. 46

## 4.5 Quantification of architectural parameters ......................................................... 47

4.5.1 Fibre bundle length ......................................................................................... 48

4.5.2 Pennation angle .............................................................................................. 48

4.5.3 Muscle volume .................................................................................................. 50

4.5.4 Physiological cross-sectional area and force index ........................................... 50

## 4.6 Sarcomere length ............................................................................................... 51

## Chapter 5 .................................................................................................................. 54

## 5 Results .................................................................................................................... 54

5.1 3D model .............................................................................................................. 54

5.1.1 3D models of muscle groups ......................................................................... 57

5.1.2 Muscles of mastication .................................................................................... 58

5.1.3 Suprahyoid muscles ....................................................................................... 60

5.1.4 Infrahyoid muscles ......................................................................................... 61

5.2 Muscle morphology and architecture .................................................................. 62

5.2.1 Muscles of facial expression ........................................................................... 62

5.2.2 Muscles of mastication .................................................................................... 70

5.2.3 Suprahyoid muscles ....................................................................................... 90

5.2.4 Infrahyoid muscles ......................................................................................... 102

## Chapter 6 ................................................................................................................... 109

## 6 Discussion .............................................................................................................. 109

6.1 Modelling ............................................................................................................. 112

6.2 Muscles of facial expression .............................................................................. 113

6.3 Muscle architecture ............................................................................................ 114

6.3.1 Muscles of mastication .................................................................................... 115

6.3.2 Suprahyoid muscles ....................................................................................... 119
6.3.3 Infrahyoid muscles ................................................................. 121

6.3.4 Comparison of architecture across muscle groups ......................... 122

6.4 Limitations .................................................................................. 124

6.5 Summary ...................................................................................... 124

Chapter 7 ........................................................................................... 125

7 Conclusions .................................................................................... 125

Chapter 8 ........................................................................................... 126

8 Future directions .............................................................................. 126

References .......................................................................................... 128

Copyright Acknowledgements ........................................................... 139
**List of Abbreviations**

- **CT**: Computed tomography
- **dtMRI**: Diffusion tensor magnetic resonance imaging
- **FBL**: Fibre bundle length
- **FE**: Finite element
- **LLSAN**: Levator labii superioris alaeque nasi
- **MRI**: Magnetic resonance imaging
- **MV**: Muscle volume
- **PA**: Pennation angle
- **PCSA**: Physiological cross-sectional area
- **SL**: Sarcomere length
# List of Tables

## Chapter 2

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table 2.1</td>
<td>Stages of the cross-bridge cycle.</td>
<td>6</td>
</tr>
<tr>
<td>Table 2.2</td>
<td>The effect of pennation angle on fibre bundle force.</td>
<td>17</td>
</tr>
<tr>
<td>Table 2.3</td>
<td>Overview of methodologies used to measure architectural parameters of supra/infrahyoid and masticatory muscles.</td>
<td>26</td>
</tr>
<tr>
<td>Table 2.4</td>
<td>Summary of architectural parameter of muscles of mastication.</td>
<td>28</td>
</tr>
<tr>
<td>Table 2.5</td>
<td>Summary of architectural parameters of suprahylid muscles.</td>
<td>30</td>
</tr>
<tr>
<td>Table 2.6</td>
<td>Summary of architectural parameters of suprahylid muscles.</td>
<td>31</td>
</tr>
<tr>
<td>Table 2.7</td>
<td>Summary of imaging studies of oro-facial and supra- and infrahyoid muscles, and quantified muscle parameters.</td>
<td>31</td>
</tr>
<tr>
<td>Table 2.8</td>
<td>Summary of thickness (mm) of muscles of expression as reported in ultrasound studies.</td>
<td>32</td>
</tr>
<tr>
<td>Table 2.9</td>
<td>Summary of parameters of muscles of mastication as reported by imaging studies.</td>
<td>33</td>
</tr>
<tr>
<td>Table 2.10</td>
<td>Change in length in suprahylid muscles during swallowing.</td>
<td>34</td>
</tr>
</tbody>
</table>

## Chapter 4

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table 4.1</td>
<td>Computed tomography scan parameters.</td>
<td>41</td>
</tr>
</tbody>
</table>

## Chapter 5

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table 5.1</td>
<td>List of modelled muscles including the number of digitized fibre bundles per muscle.</td>
<td>54</td>
</tr>
</tbody>
</table>
Table 5.2. Architectural parameters of the muscles of the oral region. 66

Table 5.3. Architectural parameters of the muscles of the nasal region. 68

Table 5.4. Architectural parameters of the muscles of orbital region and scalp. 70

Table 5.5. Architectural parameters of temporalis and its parts. 72

Table 5.6. Summary of fibre bundle attachment sites in each lamina (1-8) of masseter. 77

Table 5.7: Architectural parameters of the right and left masseter muscles as a whole, superficial and deep heads, and individual laminae. 80

Table 5.8. Architectural parameters of the medial pterygoid. 84

Table 5.9. Architectural parameters of the lateral pterygoid. 86

Table 5.10. Summary of mean architectural parameters of muscles of mastication. 87

Table 5.11. Architectural parameters of digastric. 91

Table 5.12. Architectural parameters of stylohyoid and its parts. 93

Table 5.13. Architectural parameters of mylohyoid and its parts. 96

Table 5.14. Architectural parameters of geniohyoid. 99

Table 5.15. Summary of architectural parameters of suprathyoid muscles and their parts. 100

Table 5.16. Summary of architectural parameters of the infrahyoid muscles and their parts. 105
# List of Figures

## Chapter 2

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>Overview of skeletal muscle structure.</td>
<td>4</td>
</tr>
<tr>
<td>2.2</td>
<td>Sarcomere structure.</td>
<td>5</td>
</tr>
<tr>
<td>2.3</td>
<td>Sarcomere in relaxed and contracted states.</td>
<td>8</td>
</tr>
<tr>
<td>2.4</td>
<td>Length-tension relationship of a frog sarcomere.</td>
<td>9</td>
</tr>
<tr>
<td>2.5</td>
<td>Sarcomere force-length curves. Blue, frog; red, human.</td>
<td>11</td>
</tr>
<tr>
<td>2.6</td>
<td>Tendon morphology in various muscles.</td>
<td>12</td>
</tr>
<tr>
<td>2.7</td>
<td>Micrograph showing collagen fibre matrix of a tendon; haematoxylin and eosin staining.</td>
<td>13</td>
</tr>
<tr>
<td>2.8</td>
<td>Tendon structure.</td>
<td>14</td>
</tr>
<tr>
<td>2.9</td>
<td>Fibre bundle arrangement and shape of various human skeletal muscle.</td>
<td>15</td>
</tr>
<tr>
<td>2.10</td>
<td>Graph showing the relationship of cosine pennation angle (θ).</td>
<td>17</td>
</tr>
<tr>
<td>2.11</td>
<td>Relationship between $(\cos \theta)^{-1}$ and pennation angle.</td>
<td>18</td>
</tr>
</tbody>
</table>

## Chapter 4

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.1</td>
<td>Workflow pipeline summarizing the steps involved in this study.</td>
<td>40</td>
</tr>
<tr>
<td>4.2</td>
<td>Aquilion ONE™ CT scanner.</td>
<td>41</td>
</tr>
<tr>
<td>4.3</td>
<td>Digitization of fibre bundles.</td>
<td>43</td>
</tr>
<tr>
<td>4.4</td>
<td>Digitization of tendinous elements.</td>
<td>44</td>
</tr>
<tr>
<td>4.5</td>
<td>Spline transformation of digitized fibre bundles.</td>
<td>47</td>
</tr>
</tbody>
</table>
Figure 4.6. Quantification of fibre bundle length.

Figure 4.7. Determination of average tangent vector (i.e. fibre bundle orientation).

Figure 4.8. 2D schematic showing computation of the line of action of a suspensory muscle.

Figure 4.9. Axioplan 2 Imaging Microscope system.

Figure 4.10. Location of photomicrographs of each biopsy.

Figure 4.12. Measurements of ten sarcomeres in series (63x objective lens).

Chapter 5

Figure 5.1. Three-dimensional model of the digitized muscles, anterior view.

Figure 5.2. Three-dimensional model of the digitized muscles, lateral view.

Figure 5.3. Three-dimensional model of the muscles of facial expression, anterior and lateral views.

Figure 5.4. Three-dimensional model of the muscles of mastication, lateral and anterior views.

Figure 5.5. Three-dimensional model of the suprahyoid muscles.

Figure 5.6. Three-dimensional model of the infrahyoid muscles, anterior views.

Figure 5.7. Three-dimensional model of orbicularis oris, anterior views.

Figure 5.8. Oral muscles surrounding the orbicularis oris (brown), anterior and lateral views.

Figure 5.9. Three-dimensional model of zygomaticus major and minor in relation to orbicularis oris, anterior view.

Figure 5.10. Three-dimensional model of the muscles of the nasal region.
Figure 5.11. Muscles of the orbital region and scalp.

Figure 5.12. Three-dimensional model of temporalis.

Figure 5.13. Three-dimensional model of the aponeurosis of temporalis.

Figure 5.14. Principal fibre bundle orientations (lines of action) of the superficial and deep parts of temporalis, lateral views.

Figure 5.15. Sarcomere length variation within the volume of temporalis.

Figure 5.16. Three-dimensional model of masseter.

Figure 5.17. Laminae of masseter.

Figure 5.18. Aponeurotic attachment sites of laminae (1-4) of the superficial head of masseter.

Figure 5.19. Aponeurotic attachment sites of laminae (5-8) of the deep head of masseter.

Figure 5.20. Architectural parameters of the superficial and deep heads of masseter.

Figure 5.21. Mean FBL of individual laminae of right masseter.

Figure 5.22. Mean PA of individual laminae of right masseter.

Figure 5.23. Principal fibre bundle orientations (lines of action) of the superficial and deep heads of masseter.

Figure 5.24. Three-dimensional model of the medial pterygoid.

Figure 5.25. Three-dimensional model of the medial pterygoid.

Figure 5.26. Principal fibre bundle orientations (lines of action) of the anterior and posterior parts of the medial pterygoid.

Figure 5.27. Three-dimensional model of the lateral pterygoid, anterolateral views.
Figure 5.28. Principal fibre bundle orientations (lines of action) of the superior and inferior heads of the lateral pterygoid.

Figure 5.29. Similarities in principal fibre bundle orientations of the parts of the muscles of mastication, lateral and anterior views.

Figure 5.30. Three-dimensional model of digastric.

Figure 5.31. Principal fibre bundle orientations (lines of action) of the anterior and posterior bellies of digastric, lateral views.

Figure 5.32. Three-dimensional model of stylohyoid, lateral view.

Figure 5.33. Principal fibre bundle orientations (lines of action) of the superficial and deep parts of stylohyoid.

Figure 5.34. Three-dimensional model of mylohyoid.

Figure 5.35. Principal fibre bundle orientations and resultant lines of action of the individual parts of mylohyoid.

Figure 5.36. Three-dimensional model of geniohyoid.

Figure 5.37. Principal fibre bundle orientations (lines of action) of the left and right geniohyoid muscles.

Figure 5.38. Similarities in principal fibre bundle orientations (lines of action) of the parts of the suprahyoid muscles, lateral and anterior views.

Figure 5.39. Three-dimensional models of the infrahyoid muscles.

Figure 5.40. Principal fibre bundle orientations (lines of action) of the superior and inferior bellies of omohyoid.

Figure 5.41. Principal fibre bundle orientations (lines of action) of the medial and lateral parts of sternothyroid.
Figure 5.42. Principal fibre bundle orientations (lines of action) of the superficial and deep parts of thyrohyoid.

Chapter 6

Figure 6.1. Comparison of fibre bundle length of muscles of mastication between the current study and previous literature. 115

Figure 6.2. Comparison of pennation angle of muscles of mastication between the current study and previous literature. 116

Figure 6.3. Comparison of muscle volume of muscles of mastication between the current study and previous literature. 117

Figure 6.4. Comparison of physiological cross-sectional area of muscles of mastication between the current study and previous literature. 118

Figure 6.5. Comparison of fibre bundle length of suprahyoid muscles between the current study and previous literature. 119

Figure 6.6. Comparison of pennation angle of suprahyoid muscles between the current study and previous literature. 120

Figure 6.7. Comparison of physiological cross-sectional area between the current study and previous literature. 121

Figure 6.8. Comparison of architectural parameters of thyrohyoid muscle between the current study and previous literature. 122

Figure 6.9. Comparison of the FBL and PCSA of muscles of mastication, and supra- and infrahyoid muscles. 123
Chapter 1

1 Introduction

The functions and synergistic activities of the oro-facial and hyoid muscles are poorly understood, despite their importance in chewing, swallowing and verbal/non-verbal communication. The organization (architecture) of the fibre bundles and their attachment sites to internal and external tendons, bone, and fascia play an important role in determining the functional characteristics of a muscle (Zajac 1989; Gans and Gaunt 1991). It is evident from the literature that the lack of a volumetric architectural database is impeding understanding of the individual functions and coordinated activities of the oro-facial and hyoid muscles.

Previous architectural studies of these muscle groups often focused on one or a small number of muscles in a group and quantified only select architectural parameters. This has resulted in a limited ability to study architectural partitioning in individual muscles and spatial relationships between muscles in the same functional group. These studies were carried out in 2D space, with fibre bundle measurements restricted to a small number of fibre bundles sampled from the superficial surface of the muscle.

More recent developments in muscle geometry capture techniques include the use of a digitizer to obtain Cartesian coordinates of fibre bundles and tendinous/aponeurotic elements, allowing volumetric reconstruction of the entire muscle in 3D space as in situ. However, this technique has not been utilized to generate a complete model/architectural database of the musculotendinous structures of the oro-facial and hyoid regions. Pilot studies have indicated that these data can be used to analyze the functional capabilities (force generating and excursion
capabilities) of a muscle (Ravichandiran et al. 2009; Li et al. 2014), and to provide a basis for finite element modelling at the fibre bundle level (Sanchez et al. 2014).

This thesis will focus on the architecture and spatial arrangements of the oro-facial and hyoid muscles. The oro-facial muscles include the muscles of facial expression and the muscles of mastication, whereas the hyoid muscles are divided by the hyoid bone into supra- and infrahyoid muscle groups. The main objective of this thesis is to digitize, model and analyze the architecture of the oro-facial and hyoid muscles to compare their force-generating and excursion capabilities. At the same time, a comprehensive database of the musculotendinous parameters of these muscles will be established for use in modelling, imaging and clinical studies.

1.1 Contents of thesis

A synopsis of the content of each of the eight chapters of this thesis is outlined below.

- **Chapter 1** provides a brief rationale and relevance of this work.

- **Chapter 2** is a review of literature, including: structure and architecture of skeletal muscle, quantification of architectural parameters, results of previous architectural studies of oro-facial and hyoid musculature, and skeletal muscle modelling.

- **Chapter 3** presents the hypothesis and specific objectives of this thesis.

- **Chapter 4** outlines the methods used to achieve the objectives.

- **Chapter 5** summarizes the results of this study. This section is divided into two main parts: 3D modelling of the oro-facial and hyoid muscles, and their architectural parameters.

- **Chapter 6** is a discussion of the results as related to the previous literature, and innovative findings of this thesis.

- **Chapter 7** outlines the conclusions of this thesis.

- **Chapter 8** discusses possible future directions of this work.
Chapter 2

2 Literature review

2.1 Introduction

Skeletal muscle, due to its contractile properties, result in mechanical force generation that is used to move or stabilize the body. In humans, skeletal muscle consists of contractile elements (fascicles or fibre bundles) and connective tissue elements (aponeuroses, tendons and coverings of the muscle fibre), together comprising about half of the body weight of an adult. The macroscopic and microscopic structures of each of the contractile and connective tissue elements determine the functional capabilities of a muscle (Zajac, 1989; Lieber and Ward, 2011).

2.2 Contractile elements

The macroscopic structure of the contractile elements will be discussed first, followed by the microscopic structure.

2.2.1 Macroscopic structure

The contractile elements have a hierarchical arrangement. Muscle fibres (muscle cell or myocytes) are the basic units of a muscle. Each muscle fibre is wrapped in a connective tissue coat, the endomysium, and grouped into fibre bundles or fascicles. Each fibre bundle is further surrounded by perimysium and grouped with other fibre bundles to form a muscle. The external connective tissue around a muscle is the epimysium (Figure 2.1).
2.2.2 Microscopic structure

Muscle fibres are specialized contractile multinucleated cells. Each muscle fibre contains myofibrils, which are composed of a series of sarcomeres. Sarcomeres are the basic contractile units of a muscle fibre, consisting of myosin filaments centrally and laterally placed actin filaments extending between the myosin filaments (Figure 2.1). When viewed using conventional light microscopy, skeletal muscle fibres exhibit a striation pattern of alternating light and dark bands (Figure 2.2A).
Figure 2.2. Sarcomere structure. A. Light and dark striation pattern of skeletal muscle fibres; light micrograph. B. Schematic illustration of the banding pattern of the actin and myosin filaments. The sarcomere is bounded by the Z-disk. Myosin filaments (orange), actin filaments (grey lines). C. Electron micrograph of banding pattern. A. A-band; H. H-zone; I. I-band; M. M-line. Histology, 7th Ed. Reproduced with permission from Dr Cormack.

The dark band, consisting of partially overlapping actin and myosin filaments, is the A-(anisotropic) band, and the light band, consisting of only actin filaments, is the I-(isotropic) band (Figure 2.2B and C). The actin filaments are anchored to the Z-disk (line), which delineates the borders of the sarcomere (Hanson and Huxley, 1953; Huxley, 1953). In the center of the A-band, there is a slightly lighter region consisting of thick filaments only, the H-zone, at the midpoint of which is the M-line (Figure 2.2B).

2.2.3 Contractile mechanism

The sliding filament theory describes the interaction between the thin actin and thick myosin filaments that results in shortening of the sarcomeres. The summation of the shortening
of each sarcomere results in muscle contraction (Huxley and Hanson, 1954; Huxley and Niedergerke; 1954; Sosa et al., 1994; Huxley, 1999). When a muscle actively contracts, it shortens and thickens.

The mechanism of sliding of the actin filament over the myosin filament is described as the cross-bridge cycle. The cross-bridge cycle consists of 4 main stages, each of which is summarized in Table 2.1.

**Table 2.1. Stages of the cross-bridge cycle.**

<table>
<thead>
<tr>
<th>Stage 1: Attachment</th>
<th>Stage 2: Release</th>
</tr>
</thead>
<tbody>
<tr>
<td>In the absence of ATP, the myosin head is tightly bound to actin.</td>
<td>The binding of ATP to the myosin head uncouples myosin from actin.</td>
</tr>
</tbody>
</table>
Stage 3: ATP is hydrolyzed; the energy released from hydrolysis bends the myosin head, causing it to advance a short distance along the actin filament.

Stage 4: The myosin head binds to actin. The release of Pi and ADP causes the myosin head to straighten, and results in movement of the thin filament along the thick filament (power stroke).

Cycle begins again at stage 1.

Histology: A Text and Atlas, 6th Ed. Reproduced with permission from Lippincott Williams & Wilkins

As muscle fibres contract, the actin filaments slide over the myosin filaments, increasing their overlap. The Z-lines defining the lateral borders of the sarcomere move closer to one another, i.e., sarcomere shortens (Figure 2.3). Shortening of the sarcomere does not result in a length change in either the thick or thin filaments. When relaxed and contracted muscle fibres are compared, in the contracted state the H-zone disappears and the length of the I-band markedly decreases, while the A-band remains the same length (Figure 2.3 C and D).
Figure 2.3. Sarcomere in relaxed and contracted states. Schematic illustration (A) and electron micrograph (B) of a relaxed sarcomere. Schematic illustration (C) and electron micrograph (D) of a contracted sarcomere. Essential Histology, 2nd Ed. Reproduced with permission from Dr Cormack.

2.2.4 Length-tension relationship

The length-tension (L-T) curve shows the relationship between the length of a muscle fibre and the maximum force that the fibre can produce at that length. This relationship is dependent on the extent of overlap between the actin and myosin filaments, i.e., sarcomere length.

The L-T relationship was determined by clamping frog muscle fibres at different sarcomere lengths while inducing isometric contraction and simultaneously recording the tension generated (Gordon et al., 1966). When the L-T relationship was plotted, it was observed that the peak isometric force a muscle fibre could generate was at a sarcomere length between 2.00-2.20μm, the optimal sarcomere length. This region is referred to as the plateau on the L-T curve (Figure 2.4 C).
Figure 2.4. Length-tension relationship of a frog sarcomere (adapted from Gordon et al. 1966). The L-T curve and relative overlap of actin and myosin filaments at key points on the curve.
When the sarcomere length is greater or less than the optimal length, the force generation capability of a muscle fibre decreases, as can be seen in the ascending (A and B) and descending (D) limbs of the L-T curve (Figure 2.4, upper part). The ascending limb can be divided into two parts: part A (1.27μm to 1.67μm) showing a rapid increase in tension as the fibre length increases; and part B (1.67μm to 2.05μm) showing a slow increase in tension as fibre length increases until the optimal sarcomere length is reached. At 2.20μm, the tension starts to decrease, forming the descending limb of the L-T curve, which continues until there is no active tension at a sarcomere length of 3.65μm.

The magnitude of the tension produced by a sarcomere is linearly related to the number of cross-bridges formed between the actin and myosin filaments, and correlates with the extent of overlap of the filaments (Figure 2.4, lower part). Gordon et al. (1966), in their original experiments, found that in frog muscle fibres, there is maximum overlap between the actin and myosin filaments at a sarcomere length of 2.20μm to 2.00μm, resulting in the greatest tension. At a sarcomere length of less than 2.00μm, interdigitation and collision between the filaments and the Z-disk may result in reduction of the effectiveness of cross-bridge formation and/or creation of forces that oppose the active shorting of the sarcomere (Sato, 1977; Huijing, 1998; Rassier et al., 1999). The myosin filament is about 1.67μm long, and the actin filament about 1.00μm; therefore, at a sarcomere length of 3.67μm, no overlap between the actin and myosin filaments occurs and thus no active tension can be generated.

Walker and Schrodt (1974) interpolated the L-T curve for human skeletal muscle fibres based on the comparison between human and frog sarcomere microstructure (Figure 2.5). In humans, the thick myosin filament was about the same length as that of the frog’s, but the thin actin filament was slightly longer (about 1.27μm in humans and 1.00μm in frog). Therefore,
based on the structural differences, the optimal length of the human sarcomere was predicted to be between 2.64 and 2.81μm, and the active operating range between 1.27 and 4.24μm (Walker and Schrodt, 1974; Rassier et al., 1999). However, no study was found that examined the L-T relationship in human muscle fibres.

Figure 2.5. Sarcomere force-length curves. Frog (blue) and human (red).

2.3 Connective tissue elements

2.3.1 Macroscopic structure

The contractile and connective tissue elements are closely associated with each other. The muscle fibres are bound to the endomysium via protein anchors (Petrof et al., 1993; Ehmsen et al., 2002). The endomysium is in turn continuous with the perimysium, which surrounds fibre bundles, and the epimysium, which surrounds the muscle as a whole. This close integration between the muscle fibres and the connective tissue network is important for the protection of cellular integrity during contraction, and effective force transmission (Street and Ramsey, 1965; Petrof et al., 1993; Jarvinen et al., 2002).
Tendons are the largest discrete connective tissue elements that serve to transmit forces from the muscle belly to bone (Benjamin et al., 2008). Depending on their location relative to the fibre bundles, tendons can be divided into internal and external parts/tendons (Figure 2.6).

Figure 2.6. Tendon morphology in various muscles. (A) Gastrocnemius. (B) Soleus with gastrocnemius reflected. (C) Extensor carpi ulnaris with superficial muscle fibres removed. (D) Masseter. (E) Temporalis. IT, internal tendon; ET, external tendon; MB, muscle belly.
An internal tendon often forms an aponeurosis, a thin sheet of dense connective tissue. The majority of the collagen fibres in an aponeurosis are arranged in parallel to permit unidirectional transmission of forces along the fibre bundles. An internal tendon is often continuous with the external tendon, the part of the tendon that lies outside of the muscle belly. The external tendon is often a thick, cord-like structure that serves to bridge the muscle belly and its attachment site.

2.3.2 Microscopic structure

Tendons are comprised of dense regular connective tissue, consisting primarily of type I collagen fibres that run in the same direction and plane (Figure 2.7).

![Figure 2.7. Micrograph showing collagen fibre matrix of a tendon; haematoxylin and eosin staining.](image)

This gives tendons great tensile strength and thus the ability to withstand the forces generated during muscle contraction. The fibroblasts are the cellular components of tendons that lie between the parallel collagen fibres.

Similar to the contractile elements in the muscle belly, the collagen in tendons is also arranged hierarchically, beginning with tropocollagen molecules that are cross-linked to form microfibrils (Figure 2.8 A).
Tropocollagen molecules are staggered in their arrangement, creating a banding pattern in the microfibril. A group of microfibrils forms a collagen fibre and multiple fibres, in turn, are grouped together to form collagen fibre bundles or fascicles (Figure 2.8 B).

### 2.4 Muscle Architecture

Muscle architecture is the arrangement of contractile and connective tissue elements within a muscle volume (Zajac, 1989; Lieber and Ward, 2011). The organization of the fibre bundles and their attachment sites to internal and external tendons, bone, and fascia play an important role in determining the functional characteristics of a muscle (Zajac 1989; Gans and Gaunt 1991).

The shape of a muscle and the arrangement of its fibre bundles vary and may be grouped into: parallel, pennate, circular or multibellied muscles (Figure 2.9). Most commonly, muscles are parallel or pennate. In parallel muscles, the fibre bundles are longitudinally oriented and extend the full length of the muscle. Parallel muscles can be sheet-like (flat; Figure 2.9 H), belt-
like (strap; Figure 2.9 E) or spindle-like (fusiform; Figure 2.9 I). Pennate muscles can be unipennate (Figure 2.9 F), resembling a half feather; bipennate (Figure 2.9 G), resembling a whole feather; or multipennate (Figure 2.9 B), where the fibre bundles lie between multiple tendons. Circular muscles act as sphincters (Figure 2.9 J), and multibellied muscles consist of two or more heads, often can be connected by intermediate tendon or tendons (Figure 2.9 A, D).

Figure 2.9. Fibre bundle arrangement and shape of various human skeletal muscle. Essential Clinical Anatomy, 5th Ed. Reproduced with permission from Lippincott Williams & Wilkins.

Quantitative studies of muscle architecture have yielded a set of parameters that specify the basic anatomical features of a musculotendinous unit, as well as provide insight into the functional characteristics of a muscle (Zajac, 1989; Lieber and Ward, 2011). These quantifiable parameters include: fibre bundle length, pennation angle, muscle volume, physiological cross-
sectional area, sarcomere length, and tendon geometry (Gans and Gaunt 1991; Zajac, 1992; Lieber and Ward, 2011). The definition and functional significance of these parameters are outlined below.

2.4.1 Fibre bundle length

Fibre bundle length (FBL) is the length of a fibre bundle between its attachment sites. In relation to muscle function, FBL influences both the excursion capacity and maximum shortening velocity of a muscle (Gans and de Vree, 1987; Zajac, 1992; Ranssier, 1999; Lieber and Ward, 2011). A muscle with longer fibre bundles will have greater excursion and speed of contraction.

This relationship between FBL and the distance and velocity of excursion of a muscle can be accounted for by the arrangement of the sarcomeres within a fibre bundle. The length of a muscle fibre, and therefore a fibre bundle, is determined by the number of sarcomeres arranged in series. Upon contraction, all of the sarcomeres in a muscle fibre shorten simultaneously and by a proportional amount (Lieber and Friden, 2000). This means that the greater the FBL, the greater the change in FBL and muscle length (excursion) during muscle contraction (Stevens et al. 2014).

2.4.2 Pennation angle

Skeletal muscles exert a linear force on their attachment sites and the direction of the resultant force vector is referred to as the muscle’s line of action. Pennation angle (PA) is the angle between a fibre bundle and the muscle’s line of action. In the literature, many synonymous terms have been used to describe the muscle’s “line of action”, including performance line (Gans and de Vree, 1987), force-generating axis (Lieber et al., 1992), and tendon axis (Gans and Bock, 1965).
Pennation angle influences the force-generating capability of a muscle (Table 2.2).

**Table 2.2. The effect of pennation angle on fibre bundle force.**

| As a fibre bundle contracts: | 1. A force vector is generated in the direction of the fibre bundle.  
2. Due to its pennation angle (θ), the force vector is broken down into x- and y-components.  
3. The force transmitted along the x component (line of action) is reduced by the cosine of the pennation angle.  
4. The y-component is perpendicular to the muscle’s line of action and does not directly contribute to the resultant force, but produces a translational shift at the muscle’s insertion site, altering the orientation of the line of action if not opposed. |

At the fibre bundle level, only a portion of the force generated by each fibre bundle is transmitted along the line of action to the muscle’s attachment site. The force transmitted by each fibre bundle is reduced by the cosine of the pennation angle (Figure 2.10). Therefore, as the pennation angle increases, the proportion of the force contributed by individual fibre bundles decreases.

**Figure 2.10. Graph showing the relationship of cosine pennation angle (θ).**
This effect is mitigated at the muscle level, since increased pennation allows more fibre bundles to be packed into a given muscle volume, at the expense of FBL and thus excursion. Due to the overall greater number of fibre bundles, pennate muscles generally have a greater force-generating capability than parallel-fibred muscles of equal volume (Gans and de Vree, 1987; Gans and Gaunt, 1991; Lieber et al., 1990; Zajac, 1992).

Pennation angle not only influences force production, but also excursion. The distance of excursion of a muscle relative to its fibre bundles is described by the reciprocal of cosine of the mean pennation angle \((\cos \theta)^{-1}\).

For example, a muscle with a mean pennation angle of:

- 50°, the excursion of the muscle is about 1.5 times that of its fibre bundles.
- 10°, the excursion of the muscle is about 1.02 times that of its fibre bundles.

The reciprocal cosine graph in figure 2.11. illustrates this relationship.

![Figure 2.11. Relationship between \((\cos \theta)^{-1}\) and pennation angle.](image)

2.4.3 Muscle volume

Muscle volume (MV) is defined as the amount of space a muscle occupies. Although volume can, on a gross level, influence a muscle’s functional capability, the force generation or
the excursion characteristics of a muscle are more closely related to its fibre bundle arrangement/geometry. For example, two muscles of an equal volume but different fibre bundle architecture can have significant differences in their force-generating or excursion capabilities.

2.4.4 Physiological cross-sectional area and force index

Physiological cross-section area (PCSA) is a comparative estimate of a muscle’s force-generating capability. Physiological cross-sectional area is defined as the sum of cross-sectional areas of all of the fibre bundles within a muscle. However, summation of the cross-sectional area of each fibre bundle is difficult, if not impossible, using direct cadaveric measurements.

Theoretically, the peak isometric force that a muscle can generate is estimated by multiplying the muscle’s PCSA with a maximum stress constant (Lemay and Crago, 1996). However, the value of this constant remains controversial, with different values reported in the literature, e.g., 35 N/cm\(^2\) (Zajac, 1992), 45 N/cm\(^2\) (Hermann and Delp, 1999), and 22.5 N/cm\(^2\) (Lieber and Belvin, 2011). In addition, there is doubt whether the maximum stress value is consistent across all human skeletal muscles (Buchanan, 1995).

Force index is a further indicator of the functional characteristics of a muscle. This index is the ratio between muscle’s PCSA and its volume, and normalizes the parameters between small and large muscles.

2.4.5 Sarcomere length

Sarcomere length (SL) is the distance between two consecutive Z-disks (lines). It is an indicator of the degree of contraction/stretch of the muscle fibres. The length-tension curve shows that muscle with a sarcomere length within the optimal range (2.64-2.81\(\mu\)m) produces the greatest isometric tension (Lieber et al. 1994).
2.4.6 Tendon geometry

Since the external tendon is usually cylindrical in shape and ellipsoidal in cross-section, the length, cross-sectional area and volume have been used to characterize its geometry (Murry et al., 2000; Langenderfer et al., 2004). In contrast, internal tendons/aponeuroses have not been extensively studied. Only a few large aponeuroses have been quantified, e.g., for masseter. Parameters studied included maximum length and width, surface area and volume (van Eidjen et al., 1997; Langenderfer et al., 2004; Cioffi et al., 2012).

Long, thin external tendons are more compliant than short, wider tendons, enabling stretch and recoil during muscle contraction or passive joint movement. Long, external tendons have been postulated to enhance the excursion of the muscle, especially during cyclic stretch-shortening activities such as running (Zajac, 1992; Biewener and Roberts, 2000). In contrast, broad internal tendons/aponeuroses, i.e., large surface area, significantly increase the overall stiffness of the muscle tissue, which can augment the muscle’s force-generating capability (Zajac, 1992; Cioffi et al., 2012).

2.5 Measurement of architectural parameters

Musculotendinous architectural parameters have most commonly been obtained from cadaveric studies, but increasingly, data have been acquired from in vivo medical imaging modalities such as ultrasound, if appropriate. When using any of these methods, the measurable architectural parameters have been quantified in 2D space, making it difficult to interpret the volumetric arrangement of fibre bundles and connective tissue elements. Cadaveric studies enable quantification of FBL, PA, MV, PCSA, and superficial tendon morphology. Each imaging modality enables documentation of only a small number of architectural parameters. For
example, ultrasound cannot be used to calculate MV but can be used to determine regional FBL and PA.

More recently, 3D methodologies have been developed to model the contractile and connective tissue elements of skeletal muscle throughout the entire muscle volume and quantify the architectural parameters of cadaveric specimens in 3D space (Kim et al., 2007; Rosatelli et al., 2008; Ravichandiran et al., 2009). Imaging technologies such as MRI and CT can capture muscle volume and the extent of some aponeuroses, but not internal fibre bundle architecture (Holzbaur et al. 2007; Smeulders et al. 2010; Cioffi et al. 2013). Diffusion tensor MRI with tractography, can capture general fibre bundle direction but not throughout the muscle volume. In addition the data-to-noise ratio is high and this technique cannot differentiate between connective and contractile elements (Froeling et al. 2012; Levin et al. 2011; Schenk et al. 2013).

In this section, the measurement techniques used in the 2D and 3D approaches will be discussed.

2.5.1 Two-dimensional measurements

2.5.1.1 Fibre bundle length

In most architectural studies, FBL was measured manually from cadaveric specimens using rulers or calipers (Sacks and Roy, 1982; Lieber et al., 1992; van Eijden et al., 1997). Only a limited number of fibre bundles (2-70) were quantified in each study. The location of sampling of fibre bundles was either from the surface of the muscle or undefined. Furthermore, the measurements were commonly taken from excised fibre bundles, increasing the possibility of change in their length from in situ (Schumacker, 1961; Weijs and Hillen, 1984). When using ultrasonography, the FBL was measured from the images using the measurement tool in the equipment, other software or manually.
2.5.1.2  Pennation angle

Pennation angle has been quantified directly from cadaveric specimens using a protractor or goniometer (Lieber et al., 1992; van Eijden et al., 1997). Pennation angle was measured at one or both attachment site(s), as the angle of the fibre bundle makes at its attachment site to aponeurosis, tendon or bone. On ultrasound scans, the angles at attachment site(s) were quantified using software or manually with a protractor (Kim et al., 2013; Kwah et al., 2013).

Although the definition of “pennation angle” states that the angle should be measured to the muscle’s line of action, this has not been possible because this line cannot be visualized on a specimen or ultrasound scan. In earlier studies, the line of action was estimated as a line joining the centre point of the origin of the muscle to the attachment site of the tendon of insertion, or was based on the estimated centre of the tendon of insertion only (Jensen and Davy, 1975; Lieber et al., 1992; Arnold and Delp, 2011).

2.5.1.3  Muscle volume

Traditionally, MV has been measured using water displacement (Mendez and Keys, 1960; Friederich and Brand, 1990). Due to the methodological difficulties of the water displacement technique, MV was derived indirectly by dividing muscle mass with a standard muscle density \((1.0597 \text{g/cm}^3)\) obtained from fresh canine and rabbit muscles (Mendez and Keys, 1960).

More recently, computed tomography (CT) and magnetic resonance imaging (MRI) have been used to determine MV, but both techniques have limitations due to the inability to capture the entire muscle when the ends are tapered or have narrow tendinous attachments (Blemker and Delp 2005; Gilles et al., 2006; Holzbaur et al., 2007; Smeulders et al., 2010).
2.5.1.4 Physiological cross-sectional area

Direct measurement of PCSA was attempted in some of the early muscle architectural studies. First, all of the fibre bundles in a muscle were removed and placed in parallel in a U-shaped trough. Then, the stack of fibre bundles was compacted gently with pressure until the density reached roughly the same level as in the original specimen. The measured cross-sectional area of the fibre bundle stack provided an estimation of the muscle’s PCSA (Buchner, 1877; Schumacher, 1966). However, the direct measurement method is complex and susceptible to error.

More commonly, PCSA is derived mathematically from the MV, mean FBL, and more recently, the mean PA using the equation below (Brand et al., 1986; Lieber et al., 1992; Zajac, 1992):

\[
PCSA \text{ (cm}^2) = \frac{\text{Muscle Volume (cm}^3) \cdot \cosine\theta}{\text{Average fibre bundle length (cm)}}
\]

Accurate PCSA estimation requires detailed knowledge of the architectural parameters of a muscle (Zajac, 1989; Lieber and Ward, 2011). However, this methodology of PCSA calculation has several limitations:

1) The equation relies on one average value for each architectural parameter, and thus does not take architectural variability within the muscle volume into consideration.

2) The mean architectural parameters have been obtained from a limited number of fibre bundles usually sampled from the superficial surface of the muscle, which can result in over-generalization of the parameters.
3) Architectural parameters have been obtained using 2D manual measurement techniques, which are subject to bias and cannot be correlated to the line of action or a specific location within the muscle volume.

2.5.1.5 Sarcomere length

Sarcomere length has been measured using light microscopy or laser diffraction. When light microscopy is utilized, a muscle biopsy is viewed using a 63 or 100x objective lens to measure the length of a striation consisting of adjacent light and dark bands (Sack and Roy, 1986; Pearson et al., 2012). When using laser diffraction, a laser beam (a coherent light source) is passed through a muscle fibre, producing a regularly spaced interference pattern. From this interference pattern, SL can be quantified (Lieber et al., 1984; Murray et al., 2000).

2.5.2 Three-dimensional measurement

Digitization and high resolution 3D modeling of cadaveric specimens enables visualization and quantification of both the contractile and connective tissue elements of a muscle in greater detail than was previously possible.

The contractile elements, i.e., the fibre bundles, are digitized throughout the entire volume of the muscle. Using this technique, depending on the size of the muscle, up to 2000 fibre bundles have been digitized (Li et al., 2014). The large number of fibre bundles digitized will capture architectural variation within the muscle volume. The density of points digitized on each fibre bundle can be increased or decreased depending on the length, complexity and curvature of the fibre bundle. Data point collection, in situ, throughout the length of the muscle fibre captures the course of the fibre bundle in much greater detail than when measuring between attachment sites in 2D.
Using digitization, it is possible to capture the relationships of the connective tissue elements, i.e., external and internal tendons/aponeuroses, to the fibre bundles. The external tendon can be reconstructed in 3D using digitized cross-sectional data, and its continuity with internal tendons documented. The internal tendons and aponeuroses can be captured to their full extent in the muscle volume.

Quantification of the architectural parameters, using the digitized Cartesian coordinate data, makes it possible to calculate the parameters in 3D space, incorporating complex geometry. Most notably, this can be done at the fibre bundle level, rather than using means of parameters. Volumetric data, such as muscle volume and PCSA, can be extracted from the digitized fibre bundle data using geometric tessellation (Lee et al., 2012).

2.6 Architecture of the oro-facial and hyoid muscles

This thesis will focus on the architecture and spatial arrangement of the oro-facial and hyoid muscles. The oro-facial muscles include the muscles of facial expression and the muscles of mastication, whereas the hyoid muscles are divided by the hyoid bone into supra- and infrahyoid muscle groups. All of these muscles work together, in a coordinated fashion, to produce facial expression, mastication, swallowing and speaking. The few previous architectural studies found in the literature were cadaveric or in vivo, using ultrasound, CT or MRI. Most of the studies were descriptive and quantified only select architectural parameters. An overview of these studies will be provided in the next sections of this thesis.

2.6.1 Cadaveric studies

Previous cadaveric studies of the oro-facial and hyoid musculature all quantified the architectural parameters in 2D. The 2D measurement techniques were discussed in section 2.5.1
of this thesis. The specific methodology used in each cadaveric architectural study of the oro-facial and hyoid musculature is summarized in Table 2.3.

**Table 2.3. Overview of methodologies used to measure architectural parameters of supra/infrahyoid and masticatory muscles.**

<table>
<thead>
<tr>
<th>Study</th>
<th>FBL</th>
<th>PA</th>
<th>Mass/volume (MV)</th>
<th>PCSA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Van Eijden et al. 1997</td>
<td>Caliper</td>
<td>Muscle longitudinal section</td>
<td>Contractile-tissue (hand dissected) weight / muscle density (1g/cm³).</td>
<td>$\frac{MV}{FBL}$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tendon plate and FBs traced on acetate paper</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>PA protractor</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weijs and Hillen, 1984</td>
<td>Manual.</td>
<td>Not measured.</td>
<td>Contractile-tissue (hand dissected) weight / muscle density (1g/cm³).</td>
<td>$\frac{MV}{FBL}$</td>
</tr>
<tr>
<td></td>
<td>FBs not in situ.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Schumacher, 1961</td>
<td>Manual.</td>
<td>Muscle longitudinal section</td>
<td>Contractile-tissue (hand dissected) weight / muscle density (1g/cm³).</td>
<td>$\frac{MV}{FBL}$</td>
</tr>
<tr>
<td></td>
<td>FBs not in situ.</td>
<td>Tendon plate and FBs on plexiglass</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>PA protractor (&lt;10° not reported)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pearson, et al. 2011, 2013</td>
<td>• Half head on 2D grid.</td>
<td>Half head on 2D grid.</td>
<td>Total muscle weight / muscle density (1g/cm³)</td>
<td>$\left(\frac{MV}{FBL}\right)\cos PA$</td>
</tr>
<tr>
<td></td>
<td>• FBL from digital image.</td>
<td>Line of action and PA from digital image.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

FBL, fibre bundle length; PA, pennation angle; MV, muscle volume; PCSA, physiological cross-sectional area.

The remainder of this section of this thesis will focus on comparing the quantified architectural parameters of the muscles of mastication, muscles of facial expression, and supra- and infrahyoid muscles reported in these studies.

2.6.1.1 **Muscles of facial expression**

The architectural parameters of the muscles of facial expression have not been investigated. Only two cadaveric studies were found that reported the mean length and width of these muscles (Balogh et al., 1988; Happak et al., 1997). Balogh et al. (1988) investigated
thirteen muscles of facial expression and Happak et al. (1997) investigated eight. Other studies were descriptive and focused on muscle shape and location of attachment (Gassner et al. 2008; Hutto and Vattoth, 2015). Thus, the architecture of the muscles of facial expression is largely unknown.

2.6.1.2 Muscles of mastication

Three cadaveric studies were found that investigated the architectural parameters of all four muscles of mastication, including masseter, temporalis, medial pterygoid and lateral pterygoid. The documented architectural parameters of each study are summarized in Table 2.4. In two studies, the architectural parameters were summarized for the muscle as a whole, whereas in the van Eijden et al. (1997) study, each muscle was further subdivided using attachment criteria or visual definition of superficial changes in fibre bundle direction. Sarcomere length and pennation angle were only measured in one of the three studies (Eijden et al., 1997); thus, it is not possible to compare these parameters using the previous literature.

When considering the results of these three studies, FBL was relatively consistent when reported for the muscle as a whole, but van Eidjen et al. (1997), studying sub-divisions of the muscles, found large differences in FBL throughout the volume of the muscle. This was most evident when comparing the superficial and deep parts of the masseter muscle.

Muscle volume, which is included in the PCSA calculation, was the most variable parameter in the three studies. For example, the average volume of masseter (24.58±4.37 cm$^3$) reported by van Eijden et al. (1997) was 3 times greater (7.99 cm$^3$) than that reported by Shumacher (1961). Physiological cross-sectional area therefore also varied; for example, in the three studies the PCSA for temporalis ranged from 3.81 cm$^2$ (Shumacher, 1961) to 13.25±3.30 cm$^2$ (van Eijden et al. 1997).
Table 2.4. Summary of architectural parameter of muscles of mastication.

<table>
<thead>
<tr>
<th>Study</th>
<th>Muscle</th>
<th>n</th>
<th>MV (cm$^3$)</th>
<th>FBL (mm)</th>
<th>SL (μm)</th>
<th>PA (°)</th>
<th>PCSA (cm$^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Van Eijden et al. 1997</td>
<td>Temporalis (anterior)</td>
<td>8</td>
<td>24.35±5.70</td>
<td>27.1±3.8</td>
<td>2.35±0.14</td>
<td>15.3±2.0</td>
<td>7.70±2.12</td>
</tr>
<tr>
<td></td>
<td>Temporalis (posterior)</td>
<td>8</td>
<td>16.55±3.23</td>
<td>25.7±3.3</td>
<td>2.31±0.12</td>
<td>11.6±1.9</td>
<td>5.55±1.27</td>
</tr>
<tr>
<td></td>
<td>Temporalis (Total)</td>
<td>8</td>
<td>40.9±8.41</td>
<td>26.4±3.4</td>
<td>X</td>
<td>X</td>
<td>13.25±3.30</td>
</tr>
<tr>
<td>Weijs and Hillen; 1984</td>
<td>Temporalis</td>
<td>6</td>
<td>20.58±7.12</td>
<td>31.0±0.43</td>
<td>X</td>
<td>X</td>
<td>7.11±2.12</td>
</tr>
<tr>
<td>Schumacher, 1961</td>
<td>Temporalis</td>
<td>30</td>
<td>12.37</td>
<td>33.34</td>
<td>X</td>
<td>X</td>
<td>3.81</td>
</tr>
<tr>
<td>Van Eijden et al. 1997</td>
<td>Masseter (superficial)</td>
<td>8</td>
<td>18.5±4.10</td>
<td>24.6±4.1</td>
<td>2.47±0.27</td>
<td>16.5±4.5</td>
<td>6.82±1.04</td>
</tr>
<tr>
<td></td>
<td>Masseter (deep)</td>
<td>8</td>
<td>12.23±1.26</td>
<td>18.0±2.8</td>
<td>2.44±0.22</td>
<td>6.7±3.2</td>
<td>3.49±0.82</td>
</tr>
<tr>
<td></td>
<td>Masseter (total)</td>
<td>8</td>
<td>24.58±4.37</td>
<td>21.3±2.9</td>
<td>X</td>
<td>X</td>
<td>10.31±1.41</td>
</tr>
<tr>
<td>Weijs and Hillen; 1984</td>
<td>Masseter</td>
<td>6</td>
<td>14.27±5.35</td>
<td>22.2±0.13</td>
<td>X</td>
<td>X</td>
<td>6.60±2.69</td>
</tr>
<tr>
<td>Schumacher, 1961</td>
<td>Masseter</td>
<td>30</td>
<td>7.99</td>
<td>25.8</td>
<td>X</td>
<td>X</td>
<td>3.02</td>
</tr>
<tr>
<td>Van Eijden et al. 1997</td>
<td>Med. Pterygoid (anterior)</td>
<td>8</td>
<td>4.16±0.89</td>
<td>13.5±1.9</td>
<td>2.48±0.36</td>
<td>12.0±3.4</td>
<td>2.47±0.57</td>
</tr>
<tr>
<td></td>
<td>Med. Pterygoid (posterior)</td>
<td>8</td>
<td>5.96±1.61</td>
<td>12.4±1.5</td>
<td>2.54±0.38</td>
<td>11.9±3.0</td>
<td>3.53±0.97</td>
</tr>
<tr>
<td></td>
<td>Med. Pterygoid (total)</td>
<td>8</td>
<td>10.12±2.26</td>
<td>12.9±1.6</td>
<td>X</td>
<td>X</td>
<td>6.00±1.24</td>
</tr>
<tr>
<td>Weijs and Hillen; 1984</td>
<td>Med. Pterygoid (total)</td>
<td>8</td>
<td>5.95±1.44</td>
<td>13.9±0.08</td>
<td>X</td>
<td>X</td>
<td>4.27±1.08</td>
</tr>
<tr>
<td>Schumacher, 1961</td>
<td>Med. Pterygoid (total)</td>
<td>30</td>
<td>3.15</td>
<td>16.02</td>
<td>X</td>
<td>X</td>
<td>1.97</td>
</tr>
<tr>
<td>Van Eijden et al. 1997</td>
<td>Lat. Pterygoid (inferior)</td>
<td>8</td>
<td>6.88±1.71</td>
<td>23.0±2.7</td>
<td>2.83±0.10</td>
<td>13.3±3.3</td>
<td>2.82±0.66</td>
</tr>
<tr>
<td></td>
<td>Lat. Pterygoid (superior)</td>
<td>8</td>
<td>2.12±0.70</td>
<td>21.1±2.2</td>
<td>2.72±0.11</td>
<td>X</td>
<td>0.95±0.35</td>
</tr>
<tr>
<td></td>
<td>Lat. Pterygoid (total)</td>
<td>8</td>
<td>9.00±1.77</td>
<td>22.2±2.2</td>
<td>X</td>
<td>X</td>
<td>3.78±0.71</td>
</tr>
<tr>
<td>Weijs and Hillen; 1984</td>
<td>Lat. Pterygoid (total)</td>
<td>6</td>
<td>6.42±1.82</td>
<td>24.2±0.27</td>
<td>X</td>
<td>X</td>
<td>2.31±1.00</td>
</tr>
<tr>
<td>Schumacher, 1961</td>
<td>Lat. Pterygoid (total)</td>
<td>30</td>
<td>3.98</td>
<td>22.02</td>
<td>X</td>
<td>X</td>
<td>1.83</td>
</tr>
</tbody>
</table>

n - number of specimens used. nFB - number of fibre bundles sampled/muscle. FBL, fibre bundle length; MV, muscle volume; PA, pennation angle; PCSA, physiological cross-sectional area; SL, sarcomere length. Med. Pterygoid - medial pterygoid. Lat. Pterygoid - lateral pterygoid. X, no available data.
Previous anatomical studies were descriptive and only provided an overview of the structure and organization of a particular muscle without quantifying architectural parameters. For example, the laminar structure of the masseter muscle was reported in several studies and was used as a morphologic variable to divide the muscle into multiple sub-volumes (Hannam and McMillian, 1994; Gaudy et al., 2000).

2.6.1.3 Suprahyoid muscles

Two cadaveric studies were found that quantified the fibre bundle architecture of the suprahyoid muscles, including digastric, mylohyoid, stylohyoid and geniohyoid (van Eijden et al., 1997; Pearson et al., 2011). One study focused on the role of the suprahyoid muscles on hyoid movement during swallowing (Pearson et al., 2011), and the other on the role of the suprahyoid muscles in elevation and depression of the mandible (Van Eijden et al., 1997). The quantified architectural parameters are summarized in Table 2.5.
Table 2.5. Summary of architectural parameters of suprahyoid muscles.

<table>
<thead>
<tr>
<th>Study</th>
<th>Muscle</th>
<th>n</th>
<th>MV (cm³)</th>
<th>FBL (mm)</th>
<th>SL (µm)</th>
<th>PA (°)</th>
<th>PCSA (cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>van Eijden et al. 1997</td>
<td>Digastric, Posterior</td>
<td>8</td>
<td>2.82 ± 0.71</td>
<td>20.5 ± 2.6</td>
<td>2.72 ± 0.14</td>
<td>14.3 ± 4.1</td>
<td>1.16 ± 0.31</td>
</tr>
<tr>
<td>Pearson et al. 2011</td>
<td>Digastric, Posterior</td>
<td>13</td>
<td>2.53 ± 0.65</td>
<td>30.27 ± 4.28</td>
<td>2.95 ± 0.43</td>
<td>7.10 ± 3.71</td>
<td>0.64 ± 0.16</td>
</tr>
<tr>
<td>van Eijden et al. 1997</td>
<td>Digastric, Anterior</td>
<td>8</td>
<td>2.72 ± 0.50</td>
<td>21.4 ± 4.5</td>
<td>2.75 ± 0.21</td>
<td>13.0 ± 6.2</td>
<td>1.16 ± 0.32</td>
</tr>
<tr>
<td>Pearson et al. 2011</td>
<td>Digastric, Anterior</td>
<td>13</td>
<td>2.37 ± 0.46</td>
<td>33.30 ± 4.50</td>
<td>2.75 ± 0.34</td>
<td>9.29 ± 3.40</td>
<td>0.55 ± 0.12</td>
</tr>
<tr>
<td>van Eijden et al. 1997</td>
<td>Mylohyoid total</td>
<td>8</td>
<td>6.07 ± 1.17</td>
<td>28.2 ± 3.3</td>
<td>2.85 ± 0.18</td>
<td>0</td>
<td>2.12 ± 0.32</td>
</tr>
<tr>
<td>Pearson et al. 2011</td>
<td>Mylohyoid (posterior)</td>
<td>13</td>
<td>2.17 ± 0.56</td>
<td>47.50 ± 3.92</td>
<td>2.43 ± 0.51</td>
<td>2.39 ± 0.85</td>
<td>0.43 ± 0.12</td>
</tr>
<tr>
<td>Pearson et al. 2011</td>
<td>Mylohyoid (anterior)</td>
<td>13</td>
<td>3.03 ± 0.47</td>
<td>32.87 ± 4.26</td>
<td>2.43 ± 0.30</td>
<td>6.99 ± 4.49</td>
<td>0.82 ± 0.18</td>
</tr>
<tr>
<td>van Eijden et al. 1997</td>
<td>Geniohyoid</td>
<td>8</td>
<td>3.41 ± 0.88</td>
<td>34.3 ± 5.1</td>
<td>2.65 ± 0.33</td>
<td>0</td>
<td>0.97 ± 0.23</td>
</tr>
<tr>
<td>Pearson et al. 2011</td>
<td>Geniohyoid</td>
<td>13</td>
<td>1.39 ± 0.46</td>
<td>35.32 ± 3.69</td>
<td>2.31 ± 0.55</td>
<td>7.30 ± 1.58</td>
<td>0.46 ± 0.16</td>
</tr>
<tr>
<td>van Eijden et al. 1997</td>
<td>Stylohyoid</td>
<td>8</td>
<td>1.5 ± 0.35</td>
<td>36.4 ± 2.8</td>
<td>2.76 ± 0.18</td>
<td>4.7 ± 0.7</td>
<td>0.39 ± 0.09</td>
</tr>
<tr>
<td>Pearson et al. 2011</td>
<td>Stylohyoid</td>
<td>13</td>
<td>2.21 ± 0.59</td>
<td>46.93 ± 7.11</td>
<td>2.80 ± 0.35</td>
<td>5.02 ± 1.83</td>
<td>0.27 ± 0.09</td>
</tr>
</tbody>
</table>

n, number of specimens; FBL, fibre bundle length; MV, muscle volume; PA, pennation angle; PCSA, physiological cross-sectional area; SL, sarcomere length.

The fibre bundle length of digastric, mylohyoid and stylohyoid varied markedly between the two studies: Pearson et al. (2011) reported a FBL up to 50 percent longer than van Eijden et al. (1997). Pennation angle also varied between the two studies; however, the PCSA reported by Pearson et al. (2011) was consistently about one half of that reported by van Eijden et al. (1997) for the digastric, mylohyoid and geniohyoid. Sarcomere length was consistent between the two studies.

2.6.1.4 Infrahyoid muscles

Infrahyoid muscles include sternohyoid, omohyoid, sternothyroid, and thyrohyoid. Pearson et al. (2013) investigated the architecture of the thyrohyoid muscle. No other studies
were found that have quantified the architecture of the other infrahyoid muscles. The FBL, PA, SL, MV and PCSA of thyrohyoid are summarized in Table 2.6.

Table 2.6. Summary of architectural parameters of suprahyoid muscles.

<table>
<thead>
<tr>
<th>Study</th>
<th>Muscle</th>
<th>n</th>
<th>MV (cm³)</th>
<th>FBL (mm)</th>
<th>SL (μm)</th>
<th>PA (°)</th>
<th>PCSA (cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pearson et al. 2013</td>
<td>Thyrohyoid</td>
<td>12</td>
<td>1.56 ± 0.49</td>
<td>30.68 ± 6.56</td>
<td>2.90 ± 0.24</td>
<td>3.78 ± 1.47</td>
<td>0.51 ± 0.18</td>
</tr>
</tbody>
</table>

n, number of specimens used; FBL, fibre bundle length; MV, muscle volume; PA, pennation angle; PCSA, physiological cross-sectional area; SL, sarcomere length.

Studies of the sternohyoid, sternothyroid and omohyoid muscles were limited to descriptions of muscle attachments and morphological variation (Leppi, 1961; Miura et al., 1995; Sonoda and Tamatsu, 2008; Nayak et al., 2009).

2.6.2 Imaging studies

Computed tomography, MRI and ultrasound have all been used to quantify a small number of architectural parameters of the oro-facial and hyoid musculature. These parameters include muscle volume, cross-sectional area, muscle thickness and percentage muscle shortening on contraction (Table 2.7). Fibre bundle length and pennation angle have not been investigated.

Table 2.7. Summary of imaging studies of oro-facial and supra- and infrahyoid muscles, and quantified muscle parameters.

<table>
<thead>
<tr>
<th>Muscle group</th>
<th>MRI</th>
<th>CT</th>
<th>Ultrasound</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MV/CA</td>
<td>% Shortening</td>
<td>Thickness</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Van Alfen et al. 2013.</td>
</tr>
<tr>
<td>Mastication</td>
<td></td>
<td>X</td>
<td>Bakke et al. 1992</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Raadsheer et al 1994</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Kubota et al. 1998.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Emshoff et al. 1999.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Raadsheer et al. 2004</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Satiroglu et al. 2005</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Raadsheer et al. 2004</td>
</tr>
<tr>
<td>Infrahyoid</td>
<td>X</td>
<td>Okada et al. 2013</td>
<td>X</td>
</tr>
</tbody>
</table>

X, no available data.
Magnetic resonance imaging studies have measured the muscle volume and cross-sectional area, CT the percentage of muscle shortening on contraction, and ultrasound muscle thickness.

### 2.6.2.1 Muscles of facial expression

The thickness of selected muscles of facial expression was measured using ultrasound. The results of the two studies are summarized in Table 2.9.

#### Table 2.8. Summary of thickness (mm) of muscles of expression as reported in ultrasound studies.

<table>
<thead>
<tr>
<th></th>
<th>Procerus</th>
<th>Zygomaticus Major</th>
<th>Levator labii superioris</th>
<th>Depressor anguli oris</th>
<th>Mentalis</th>
<th>Orbicularis oris (pars labialis)</th>
<th>Orbicularis oris (pars marginalis)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Satiroglu et al. 2005</td>
<td>3.44±0.40</td>
<td>3.48±0.49</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Van Alfen et al. 2013</td>
<td>0.56 (0.4-0.9)</td>
<td>2.5 (2.1-2.9)</td>
<td>1.6 (0.9-2.3)</td>
<td>3.0 (2.1-3.9)</td>
<td>3.0 (1.4-4.2)</td>
<td>2.2 (1.7-3.1)</td>
<td>1.5 (1.1-2.4)</td>
</tr>
</tbody>
</table>

These were the only studies that were found that quantified the architectural parameters of the muscles of facial expression. Satiroglu et al. (2005) studied 47 adult subjects, whereas van Alfen et al. (2013) studied 13 subjects. Besides the muscles listed in Table 2.8, van Alfen et al. (2013) also attempted to quantify the thickness of other facial muscles, but found that they were either too thin or indistinguishable from the adjacent muscles/soft tissue.

### 2.6.2.2 Muscles of mastication

The quantified architectural parameters of the muscles of mastication from three MRI and six ultrasound studies are summarized in Table 2.9.
Table 2.9. Summary of parameters of muscles of mastication as reported by imaging studies.

<table>
<thead>
<tr>
<th>Muscle</th>
<th>MV (cm$^3$)</th>
<th>CA (cm$^2$)</th>
<th>Thickness (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temporals (X)</td>
<td></td>
<td>(1) 5.21±0.62</td>
<td>(7) 1.82±0.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(8) 13.5-15.7</td>
<td></td>
</tr>
<tr>
<td>Masseter (3)</td>
<td>28.8±7.7</td>
<td>(1) 5.33±1.49</td>
<td>(4) Relaxed: 12.09±2.27</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(2) 5.76±1.11</td>
<td>Contracted: 14.36±2.08</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(5) Relaxed: 13.3 to 12.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Contracted: 15.4 to 16.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(6) Relaxed: 15.8±3.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Clinched: 16.7±2.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(7) Anterior: 2.72±5.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Deep: 4.53±12.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(9) Relaxed 13.56±1.95</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Contracted 14.57±1.83</td>
</tr>
<tr>
<td>Med. Pterygoid</td>
<td>X</td>
<td>(1) 3.70±0.62</td>
<td>X</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(2) 3.48±0.88</td>
<td></td>
</tr>
<tr>
<td>Lat. Pterygoid</td>
<td>X</td>
<td>(1) 4.22±0.48</td>
<td>X</td>
</tr>
</tbody>
</table>

MV, muscle volume; CA, cross-sectional area. Number in parentheses correspond to references listed in Table 2.7.

For masseter and temporalis, the reported parameters were consistent between the studies, except that of Emshoff et al. (1999). The authors suggested that the results of this study varied significantly from others due to the site of measurement. Cioffi et al. (2012), segmented the aponeuroses/tendons of the masseter and found their combined volume to be 2.0±0.8 cm$^3$.

2.6.2.3 Supra- and infrahyoid muscles

Only one imaging study, dynamic CT, was found that investigated the percentage of shortening of selected supra- and infrahyoid muscles (Okada et al., 2013). Pre- and post-contraction muscle lengths were compared (Table 2.10). The posterior digastric shortened the least (13.6 ± 8.1%), whereas the mylohyoid shortened the most, by about one-third of its length (31.6 ± 9.6%).
Table 2.10. Change in length in suprahypoid muscles during swallowing.

<table>
<thead>
<tr>
<th>Suprahypoid:</th>
<th>Max muscle length (mm)</th>
<th>Min muscle length (mm)</th>
<th>Shortening length (mm)</th>
<th>% shortening</th>
</tr>
</thead>
<tbody>
<tr>
<td>Digastric, Posterior</td>
<td>85.2 ± 8.2</td>
<td>72.6 ± 9.4</td>
<td>11.7 ± 7.2</td>
<td>13.6 ± 8.1</td>
</tr>
<tr>
<td>Digastric, Anterior</td>
<td>44.7 ± 7.5</td>
<td>36.5 ± 6.5</td>
<td>8.2 ± 4.7</td>
<td>17.9 ± 9.4</td>
</tr>
<tr>
<td>Mylohyoid</td>
<td>42.3 ± 11.3</td>
<td>29.0 ± 9.3</td>
<td>13.3 ± 5.2</td>
<td>31.6 ± 9.6</td>
</tr>
<tr>
<td>Geniohyoid</td>
<td>32.5 ± 5.5</td>
<td>22.8 ± 3.8</td>
<td>9.7 ± 4.1</td>
<td>29.2 ± 9.0</td>
</tr>
<tr>
<td>Stylohyoid</td>
<td>59.3 ± 12.3</td>
<td>46.6 ± 11.6</td>
<td>12.8 ± 7.2</td>
<td>21.3 ± 10.8</td>
</tr>
</tbody>
</table>

| Infrahypoid:                     |                        |                        |                        |              |
| Thyrohyoid                       | 30.6 ± 7.4             | 22.1 ± 6.6             | 8.5 ± 3.8              | 28.1 ± 11.7  |

Two in vivo ultrasound studies were identified that investigated muscle thickness. Emshoff et al. (1999) found that the mean thickness of the anterior digastric was 4.48±1.09mm, almost double that of the posterior digastric (2.37±0.55mm), whereas Raasheer et al. (2004) reported that the thickness of the anterior digastric ranged from 5.8-6.7mm.

2.7 Muscle modelling

Computer modeling of skeletal muscles has been used to study muscle function for many decades. Line segment models were the first to be developed, followed by volumetric models. These models form the core for dynamic simulation.

2.7.1 Line segment models

The simplest muscle models consist of a single line segment that connects the centres of the muscle’s attachment sites. This line segment is intended to capture the muscle’s line-of-action. However, a single line segment is not sufficient to represent muscles with broad attachments and/or regions that vary architecturally (Blemker & Delp 2005). To overcome this shortcoming, several line segments have been used to represent the lines of action within a
muscle. For example Hannam et al. (2003) modelled the masseter muscle using two line segments, one representing the line of action of the superficial head and the other the deep head.

These simple line models can be used as actuators to perform dynamic simulations. The actuators are used to constrain movement by programming them with basic architectural parameters, e.g., PCSA and FBL (often from a limited number of fibre bundles). However, due to the simplicity of these line segment models, they are limited in their ability to capture the contractile behaviour of muscles, especially pennate muscles with complex fibre bundle geometry (Blemker and Delp, 2005).

2.7.2 Volumetric muscle models

These 3D models can consist of:

- An empty surface shell (obtained using CT or MRI reconstruction);

- A surface shell containing generalized fibre bundle templates - an “anatomical-based approximation” of fibre bundle geometry; or

- The entire musculoaponeurotic geometry of a muscle based on digitized fibre bundle and tendon/aponeurosis data.

These volumetric models can form the core for dynamic models. The empty shell muscle models can be used to emulate, but not simulate, muscle deformation, and are limited to computer animation (Lewis et al., 2000; Kahler et al., 2001). Shell models with generalized fibre bundle templates can be transformed into dynamic finite element (FE) models (Blemker and Delp 2005; Rohrle and Pullan 2007; Cotin, 2008). However, since the generalized fibre bundle templates are not based on actual muscle geometry, the fidelity of the simulations has been
questioned.

The fidelity of dynamic simulations is dependent on the accuracy of the underlying muscle architectural data (Blemker et al., 2007; Lee et al., 2009). Rohler and Pullan (2007) stated that “although it would be desirable to base the [finite element] model fibre geometry on measurements of actual muscle fibres, it was not possible to find applicable data”. With advances in technology, it is now possible to capture fibre bundle geometry and organization using digitization (Kim et al. 2007; Rosatelli et al. 2008; Li et al. 2013). Finite element techniques using fibre bundle data are currently being developed. Sanchez et al. (2014) stated “the significant differences observed in net forces produced as the fibre field is varied, as well as discrepancies in geometric muscle deformation, suggest that we may need to pay closer attention to subtleties in the fibre architecture”, and furthermore, “… it may not be sufficient to assume some simplified fibre orientations as is common practice.”

2.8 Summary

The function and synergistic activity of the oro-facial and hyoid muscles are poorly understood despite their importance in chewing, swallowing and verbal/non-verbal communication. It is evident from the literature that the lack of a volumetric musculotendinous database is impeding progress in advances in understanding the individual functions and coordinated activity of the oro-facial and hyoid muscles.

Previous architectural studies of these muscle groups often focused on one or a small number of muscles in a group and quantified only select architectural parameters. This has resulted in a limited ability to study architectural partitioning in individual muscles and spatial relationships between muscles in the same functional group. These studies were carried out in 2D space with fibre bundle measurements restricted to a small number of fibre bundles sampled
from the superficial surface of the muscle. These manual measurements were made using tools such as calipers, protractors and grid based photography, all with inherent human error.

More recent developments in muscle geometry capture techniques include the use of a digitizer to obtain Cartesian coordinates of fibre bundles and tendinous/aponeurotic elements, allowing volumetric reconstruction of the entire muscle in 3D space. However, this technique has not been utilized to generate a complete model/architectural database of the musculotendinous structures of the oro-facial and hyoid regions. Pilot studies have indicated not only that these data can be used to analyze a muscle’s functional capabilities in more depth than was previously possible, but also that they can provide a basis for finite element modeling at the fibre bundle level.

Therefore, the main objective of this thesis is to digitize, model and analyze the architecture of the oro-facial and hyoid muscles to be able to compare their force generating and excursion capabilities. At the same time, a comprehensive database of the musculoskeletal parameters of these muscles will be established for use in modelling, imaging and clinical studies.
Chapter 3

3 Objectives and hypothesis

3.1 Objectives

Overall:

To digitize, model and analyze the architecture of the oro-facial and hyoid muscles to compare their force-generating and excursion capabilities, as determined by the muscle architectural parameters, at the muscle group, whole muscle, and muscle partition levels.

Specific:

1. To digitize the fibre bundles and tendons of the oro-facial and hyoid musculature of one cadaveric specimen.

2. To create 3D volumetric models of the oro-facial and hyoid musculature from the digitized data.

3. To reconstruct 3D surface meshes of the skeleton of the head and neck from the CT scans of the same specimen.

4. To combine the volumetric models of the muscles and 3D surface meshes of the skeleton of the head and neck to create a comprehensive musculoskeletal model.

5. To quantify the fibre bundle length, pennation angle, muscle volume, and physiological cross-sectional area of individual muscles at the fibre bundle level, and obtain SL measurements throughout the muscle volume.
6. To characterize the spatial relationships between the fibre bundles and the tendinous/aponeurotic elements of each muscle.

7. To develop preliminary observations about the force-generating and excursion capabilities of the oro-facial and hyoid musculature that will inform the development of future work adopting empirical approaches to identifying functional differences between muscle groups, muscles as a whole, and partitions within muscles.

3.2 Hypothesis
The excursion and relative force-generating capabilities, as determined by the muscle architectural parameters, will differ between the oro-facial and hyoid musculature at the muscle group, whole muscle, and muscle partition levels.
Chapter 4

4  Material and Methods

One formalin embalmed human cadaveric specimen male in early 80s with normal muscle mass and no visible signs of musculoskeletal abnormality or previous surgery was included in this study. Ethics approval was received from the University of Toronto Health Sciences Research Ethics Board (Protocol Reference #27210 and #28530) and the Mount Sinai Hospital Research Ethics Board (Protocol Reference 12-0252-E).

The methodologies used in this thesis are summarized in the flowchart below (Figure 4.1), which provides a step-by-step guide to the workflow used in this study.

![Figure 4.1. Workflow pipeline summarizing the steps involved in this study.](image-url)
4.1 Scanning of specimen

The specimen was scanned using an Aquilion ONE™ Computed Tomography (CT) scanner (Toshiba Medical Systems Corporation, Tokyo, Japan). The axial images were reformed in the sagittal and coronal planes to provide datasets in all planes.

![Aquilion ONE™ CT scanner](image)

**Figure 4.2. Aquilion ONE™ CT scanner (Toshiba Medical Systems Corporation, Tokyo, Japan).**

The scanned images were saved and exported in compressed Digital Imaging and Communications in Medicine (DICOM) format. The slice thickness was 2mm; other parameters are summarized in Table 4.1.

<table>
<thead>
<tr>
<th>Parameter (units)</th>
<th>Coronal†</th>
<th>Sagittal†</th>
<th>Axial</th>
</tr>
</thead>
<tbody>
<tr>
<td>Voltage (kV)</td>
<td>120.0</td>
<td>120.0</td>
<td>120.0</td>
</tr>
<tr>
<td>Current (mA)</td>
<td>430.0</td>
<td>430.0</td>
<td>430.0</td>
</tr>
<tr>
<td>Thickness of slice (mm)</td>
<td>3</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>DFOV (cm)</td>
<td>41.8 × 41.8</td>
<td>41.8 × 41.8</td>
<td>25.0 × 25.0</td>
</tr>
<tr>
<td>Resolution (pixels)</td>
<td>856 × 856</td>
<td>856 × 856</td>
<td>512 × 512</td>
</tr>
<tr>
<td>Window</td>
<td>350 × 3500</td>
<td>3500 × 350</td>
<td>3500 × 350</td>
</tr>
</tbody>
</table>

DFOV, display field of view.

† Images obtained in the coronal and sagittal planes were reformed from scans in the axial plane.
The skeletal elements of the head and neck region were segmented from the CT scans and volumetrically reconstructed using AMIRA (FEI Visualization Sciences Group, Bordeaux, France and Zuse Institute Berlin, Berlin, Germany).

4.2 Dissection and digitization

The specimen was placed supine, in the anatomical position, on a dissection table and the head and neck were stabilized using minimal expansion polyurethane foam (Great Stuff™, Dow Chemical Co, Midland, Michigan, USA). The foam casing prevented movement of the specimen during dissection and digitization. Prior to dissection and digitization, three screws were placed in bone as reference markers that defined a virtual 3D Cartesian coordinate space in which digitized data could be recorded and reconstructed. The screws were placed in the right and left clavicles and in the frontal bone. The skin and fascia of the face and neck were removed to expose the muscles. Digitization was carried out using a MicroScribe™ MX Digitizer (0.05mm accuracy; Immersion Corporation, San Jose, CA), as seen in Figure 4.3 A. Fibonacci, the software used to collect the data from the digitizer was developed with our computer science collaborators and named Fibonacci. The fibre bundles and tendinous elements of each of the muscles of facial expression, mastication, suprahyoid and infrahyoid were digitized using the processes outlined in the next sections.

4.2.1 Digitization of fibre bundles

Each muscle was exposed individually beginning with superficial muscles. The fibre bundles on the exposed surface of the muscle were meticulously cleaned and delineated so that each fibre bundle could be traced in its entirety. Each fibre bundle was digitized at 3-5mm intervals between attachment sites (Figure 4.3 B and C). The number of points digitized along a fibre bundle depended on its length and the complexity of its course. Next, the digitized fibre
bundles were excised to reveal the underlying fibre bundles. This process of dissection and digitization of each fibre bundle was continued throughout the muscle volume.

Figure 4.3. Digitization of fibre bundles. A. Microscribe™ MX Digitizer. B. Digitized fibre bundles of masseter specimen. Data points are purple and the points for each fibre bundle are joined by an orange line. C. Digitized fibre bundles of masseter as seen on screen using Fibonacci.

4.2.2 Digitization of tendinous elements

During digitization of the fibre bundles, all internal and external tendons, including aponeuroses, were digitized beginning with the perimeter, followed by collagen fibre bundles on the exposed surface (Figure 4.4). The digitized points were spaced at 3-5mm intervals. This process was continued during serial dissection and digitization of the fibre bundles throughout the volume of the muscle.
If external tendons were long and tubular, the periphery of the tendons was digitized to their attachment sites. Next, the tendons were marked transversely at 5mm intervals with lines which were digitized in situ before sectioning of the tendon at these sites. When the tendon had been sectioned, the circumference was digitized at each interval. The circumferential digitized data were used to reconstruct the tendon volumetrically.

### 4.3 Digitization of oro-facial and hyoid musculature

#### 4.3.1 Muscles of facial expression

To expose the superficial muscles of facial expression, a shallow midline incision was made from the bregma superiorly to the sternum inferiorly and then along the clavicles. The skin was carefully reflected laterally and the muscle fibre bundles were exposed in the superficial fascia. Dissection was very time consuming as the fibre bundles were difficult to delineate from the fascia. The fibre bundles of the muscles were digitized from superficial to deep using the
process described above. The buccinator was the deepest muscle and was therefore digitized last. All muscles of facial expression were digitized bilaterally except for buccinator and temporoparietalis. The bilaterally digitized muscles included risorius, platysma, zygomaticus major/minor, nasalis, levator labii superioris alaeque nasi (LLSAN), levator labii superioris (LLS), orbicularis oculi, levator anguli oris (LAO), depressor anguli oris (DAO), depressor labii inferioris (DLI), mentalis, orbicularis oris, frontalis, procerus, and corrugator.

4.3.2 Muscles of mastication

Since the muscles of facial expression were removed as they were digitized, the masseter with the overlying facial blood vessels, the parotid gland and duct, and fascia was exposed bilaterally. The overlying structures were removed, revealing the superficial fibre bundles and aponeurosis of masseter. Both the fibre bundles and tendinous elements were serially dissected and digitized using the process described above. Next, the zygomatic arch and temporal fascia were removed to expose the temporalis, which was then dissected and digitized. To access the lateral and medial pterygoid muscles for digitization, the coronoid process of the mandible was excised and the ramus of the mandible was removed in small fragments, exposing the medial pterygoid. The head of the mandible was left intact along with the lateral pterygoid. The temporalis, medial and lateral pterygoid were digitized on the right side only. During dissection and digitization of the muscles of mastication, the surfaces of the underlying skeletal elements, including the head and ramus of the mandible, zygomatic arch, lateral surface of the cranium and lateral pterygoid plate, were digitized as they were exposed.

4.3.3 Supra- and infrahyoid muscles

Since the platysma was digitized with the muscles of facial expression, the supra- and infrahyoid muscles had already been exposed. Any remaining fascia and blood vessels were
removed. The suprahyoid muscles were digitized first, beginning with the anterior and posterior bellies of digastric, followed by stylohyoid, mylohyoid and geniohyoid. Next, the infrahyoid muscles, including sternohyoid and omohyoid superficially and the deeper sternothyroid and thyrohyoid, were digitized.

4.4 Three-dimensional modelling of digitized data

The digitized musculotendinous data were imported into Autodesk® Maya® (Autodesk Inc., San Rafael, California) using custom software plug-ins developed in our laboratory.

The modelling process is outlined below:

- The digitized points of each fibre bundle were connected by line segments. The path of each digitized fibre bundle was approximated by a cubic uniform B-Spline curve, with clamped boundary conditions, and then stored as a Maya® NURBS curve (Ravichandiran et al. 2009). Each NURBS curve, i.e. fibre bundle, was volumetrically reconstructed into a cylindrical tube.

- The digitized points of the collagen fibre bundles along the perimeter and over the surface of the tendinous elements were connected by line segments. Similar to the fibre bundle, the digitized collagen fibre bundles were transformed into NURBS curves. The NURBS curves, i.e., collagen fibre bundles, were lofted into a 3D surface.

- To reconstruct the external tendon in 3D, the sites of the incrementally marked curves along the tendon were used to spatially locate the corresponding circumference data. The curves of the circumferential data were then lofted into a 3D surface.

- The lofted internal/external tendons and aponeuroses were combined with the cylindrically reconstructed muscle fibre bundles into one 3D model of each musculotendinous unit.

- The skeletal elements of the head and neck were volumetrically reconstructed from the CT scans and registered with the digitized surfaces of the skeletal elements.
All of the muscle models were combined with the CT based skeletal model using the digitized reference frames to create a complete, volumetric model of the oro-facial and hyoid regions.

4.5 Quantification of architectural parameters

Architectural parameters were quantified from the digitized data using custom software (Lee et al., 2012, Li et al., 2014, and Lee et al., 2014). In preparation for quantification of the architectural parameters, each digitized fibre bundle was:

1. Represented by a polyline, where the digitized points were joined by line segments (Figure 4.5 A);

2. Transformed into a smooth curve using the catmull-rom spline function to better approximate curvature (Figure 4.5 B); and

3. Uniformly resampled to increase the density of points along its length (Figure 4.5 C).

Figure 4.5. Spline transformation of digitized fibre bundles. A. Polyline connecting digitized points (red). B. Smoothing of digitized fibre bundle. C. Uniformly resampled points (blue).

The quantified architectural parameters include fibre bundle length, pennation angle, muscle volume, and physiological cross-sectional area.
4.5.1 Fibre bundle length

Fibre bundle length was obtained by summating the distances between the resampled points (Figure 4.6.)

\[ \sum_{i=1}^{n} d_{i} \]

Figure 4.6. Quantification of fibre bundle length. \( d_{i} \) represents the distance between two adjacent points on the fibre bundle.

4.5.2 Pennation angle

Pennation angle is the angle between the fibre bundle and the muscle’s line of action. The calculation of pennation angle is a three step process. Each step is outlined below.

1. The average tangent vector, i.e., fibre bundle orientation, was computed from a series of tangent vectors at each point along the distal end of the fibre bundle (Figure 4.7).
Figure 4.7. Determination of average tangent vector (i.e. fibre bundle orientation). Tangent vectors through each point along the distal end of the fibre bundle (green); averaged tangent vector (red).

2. a) Determination of the line of action for the muscle as a whole or its regions. The line of action was the vector determined by averaging the average tangent vectors of each fibre bundle within the muscle or region of interest.

2. b) For “suspensory muscles”, which are usually somewhat V-shaped, another method is used to calculate the line of action, as it is not in the direction of the fibre bundles. The muscle is transversely divided along its midline and then the average tangent vector of each fibre bundle is determined at this point. These vectors are averaged to determine the muscle’s line of action (Figure 4.8).
3. The pennation angle of each fibre bundle is computed as the angle between the fibre bundle and the line of action using the formula below:

\[ PA^i = \cos^{-1}(\text{orientation of } FB^i \cdot \text{line of action}). \]

4.5.3 Muscle volume

To calculate muscle volume, each fibre bundle was orthogonally sectioned along its length at 1-3mm intervals. At each section, the cross-sectional area of each fibre bundle was determined relative to its neighbours using Voronoi’s tessellation. The cross-sectional area of each fibre bundle at each section was averaged to determine the mean cross-sectional area of the fibre bundle. Next, to quantify the volume of each fibre bundle, its mean cross-sectional area was multiplied by its FBL. The volume of the entire muscle was computed as the sum of volumes of all of its fibre bundles.

4.5.4 Physiological cross-sectional area and force index

To calculate the physiological cross-sectional area of a fibre bundle, its mean cross-sectional area was multiplied by the cosine of its pennation angle measured relative to the line of
action. For the whole muscle, PCSA was computed as the sum of PSCAs of all of its fibre
bundles. The algorithm used to calculate PCSA was:

\[
PCSA = \sum_{i=1}^{n} \overline{A}_i \cos(\text{PA}_i)
\]

where \( \overline{A}_i \) is the mean cross-sectional area of \( FB^i \), \( n \) is the number of fibre bundles within the
muscle, and \( \text{PA} \) is the pennation angle.

Force index is the ratio between the PCSA and volume of a muscle (Woittiez et al., 1986)
and is calculated using the formula below:

\[
I = \frac{PCS}{V^{2/3}}
\]

The force index normalizes the PCSA with MV.

### 4.6 Sarcomere length

Depending on the size and complexity of the muscle, 3-15 fibre bundle biopsies were
taken throughout the muscle volume. The biopsies, 10-15mm in length, were excised from the
middle of the fibre bundles (Figure 4.6 A) and wet-mounted onto glass slides (GoldLine
Microscope Slides, VWR® International, Radnor, PA, USA) with glycerol. The biopsies were
handled with care to prevent stretching or damage. Next, each slide was covered with a No1
(0.16mm thickness) micro cover glass (VWR® International, Radnor, PA, USA) and sealed
suing Cytoseal™ 280 mounting media (Richard-Allan Scientific™, Kalamazoo, MI, USA).

Each slide was viewed with an Axioplan 2 Imaging Microscope system (Carl Zeiss
MicroImaging GmbH, Jena, Germany) using an oil immersion 63X/1.40 objective lens and
photographed with an AxioCam High Resolution digital microscope camera (Figure 4.9).
Figure 4.9. Axioplan 2 Imaging Microscope system.

For each biopsy, a photomicrograph was taken at the top left, middle and lower right regions of the slide (Figure 4.10 A). If the biopsy was narrow, two micrographs were taken, one in the top half of the slide and the other in the lower half (Figure 4.11 B).

Figure 4.10. Location of photomicrographs of each biopsy. A. Three locations in wider biopsies. B. Two locations in narrow biopsies.
Three muscle fibres, located at the top, middle and lower part of each micrograph were selected for sarcomere length measurement (Figure 4.12). First, the length of ten sarcomeres in series was measured, and then divided by ten to determine the average length of each sarcomere.

![Image of muscle fibres with measurements](image)

**Figure 4.12.** Measurements of ten sarcomeres in series (63x objective lens). The three locations of measurement at the top, middle and lower part of the micrograph are shown.

The measurements were taken with an Image J system (http://rsbweb.nih.gov/ij) further enhanced with the Bio-Formats plug-in (University of Dundee & Open Microscopy Environment, http://www.openmicroscopy.org/site/products/bio-formats).
Chapter 5

5 Results

5.1 3D model

Three-dimensional volumetric models of the muscles of facial expression, muscles of mastication, supra- and infrahyoid muscles were reconstructed from the in situ digitized data. In total, 48 muscles were digitized and modelled. Fourteen muscles were digitized unilaterally and 17 bilaterally. The number of fibre bundles digitized is dependent on the size of the muscle, as can be seen in Table 5.1. The smallest number of fibre bundles digitized was in risorius and the largest in masseter.

Table 5.1. List of modelled muscles including the number of digitized fibre bundles per muscle.

<table>
<thead>
<tr>
<th>Muscles of facial expression</th>
<th>nFB</th>
<th>Muscles of mastication</th>
<th>nFB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frontalis (B)</td>
<td>63</td>
<td>Masseter (B)</td>
<td>1265</td>
</tr>
<tr>
<td>Corrugator supercili (B)</td>
<td>43</td>
<td>Temporalis (R)</td>
<td>890</td>
</tr>
<tr>
<td>Procerus (B)</td>
<td>35</td>
<td>Medial pterygoid (R)</td>
<td>789</td>
</tr>
<tr>
<td>Orbicularis oculi (B)</td>
<td>119</td>
<td>Lateral pterygoid (R)</td>
<td>349</td>
</tr>
<tr>
<td>Nasalis (B)</td>
<td>45</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LLSAN (B)</td>
<td>72</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Levator labii superioris (B)</td>
<td>124</td>
<td>Digastric (B)</td>
<td>410</td>
</tr>
<tr>
<td>Levator anguli oris (B)</td>
<td>71</td>
<td>Mylohyoid</td>
<td>373</td>
</tr>
<tr>
<td>Zygomaticus major (B)</td>
<td>45</td>
<td>Stylohyoid (R)</td>
<td>54</td>
</tr>
<tr>
<td>Zygomaticus minor (B)</td>
<td>47</td>
<td>Geniohyoid (B)</td>
<td>111</td>
</tr>
<tr>
<td>Risorius (B)</td>
<td>22</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Depressor labii inferioris (B)</td>
<td>225</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Depressor anguli oris (B)</td>
<td>122</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Platysma (B)</td>
<td>144</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Auricularis (R)</td>
<td>56</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Buccinator (R)</td>
<td>262</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mentalis (R)</td>
<td>144</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Orbicuaris Oris</td>
<td>263</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transversus menti</td>
<td>24</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

LLSAN, levator labii superioris alaeque nasi. For bilaterally digitized and modelled muscles (B), the average number of fibre bundles for both left (L) and right (R) muscles is reported.
The integrated model shows the relationships of the musculotendinous elements of the muscle groups and their relative attachment sites to the underlying skeleton. In the anterior view, the most superficial muscles, i.e., the muscles of facial expression, overlie the deeper muscles of mastication and hyoid muscles (Figure 5.1).

**Figure 5.1.** Three-dimensional model of the digitized muscles, anterior view.
In the lateral view, the muscles of facial expression are not as numerous, permitting visualization of some of the underlying muscles of mastication and supra- and infrahyoid muscles (Figure 5.2).

Figure 5.2. Three-dimensional model of the digitized muscles, lateral view.
The 3D models are interactive and can be viewed as a composite or as individual muscle components, i.e., from individual muscles to muscle groups to combinations of muscle groups.

5.1.1 3D models of muscle groups

The 3D models of the muscle groups (muscles of facial expression, muscles of mastication, supra- and infrahyoid muscles) are presented in this section of the thesis. Within each group, the spatial relationships and attachments of the muscles relative to one another can be visualized.

5.1.1.1 Muscles of facial expression

Thirty-three muscles of facial expression are included in the 3D model (Figure 5.3).

Figure 5.3. Three-dimensional model of the muscles of facial expression, anterior and lateral views. Individual muscles are colour-coded.
When observing this model, the relationships between specific muscles in a group can be noted. For example, some observations include (Figure 5.3):

- The extent of interdigitation of the fibre bundles of frontalis (green), procerus (orange) and orbicularis oculi (orange).
- The zygomaticus minor (light green) fibre bundles lie adjacent and parallel to the inferior fibre bundles of the orbital part of orbicularis oculi.
- The asymmetry in the size and shape of the left and right muscles of facial expression, i.e., platysma (blue), orbicularis oculi (brown), and levator/depressor anguli oris (purple and magenta).
- The extent of interdigitation of the fibre bundles of the oral musculature at the angle of the mouth, i.e., orbicularis oris (aqua), buccinator, levator/depressor anguli oris, and zygomaticus major.

5.1.2 Muscles of mastication

Various views of the 3D model of the muscles of mastication are shown in Figure 5.4. The directions of the fibre bundles provide evidence of the functional similarities and differences of these muscles. The relationship of the more superficially located masseter and temporalis muscles is visualized in Figure 5.4 A to D. The deeply lying muscles of the infratemporal fossa, the medial and lateral pterygoids, are modelled in Figure 5.4 E and F.
Figure 5.4. Three-dimensional model of the muscles of mastication, lateral and anterior views. A and B. Muscles of mastication with the underlying skeletal elements. C-F. Muscles of mastication without the underlying skeletal elements.
5.1.3 Suprahyoid muscles

The 3D model of the suprahyoid muscles is presented in Figure 5.5. From the 3D model, the spatial relationships of the individual muscles and the skeletal elements can be visualized. The extent of mylohyoid and the overlapping belly of the anterior digastric is evident in this model, along with the location of the intermediate tendon of digastric piercing the belly of stylohyoid laterally and emerging from its medial surface, just superior to the hyoid bone. Also, the variable length of the fibre bundles of mylohyoid and their orientation are highlighted in Figure 5.5 C.

Figure 5.5. Three-dimensional model of the suprahyoid muscles. A. Inferior view. B and C. Lateral views.

5.1.4 Infrahyoid muscles

The infrahyoid muscles are longitudinally oriented strap muscles of variable length, depending on their attachment sites (Figure 5.6). The fibre bundles of these muscles, including omohyoid, are arranged in parallel. The medial part of sternothyroid lies almost completely deep to sternohyoid, whereas the lateral part is exposed (Figure 5.6 A).

Figure 5.6. Three-dimensional model of the infrahyoid muscles, anterior views. H, hyoid; TC, thyroid cartilage (partially segmented); dashed line, superior border of manubrium. A. Superficial muscles. B. Deep muscles.

5.2 Muscle morphology and architecture

5.2.1 Muscles of facial expression

5.2.1.1 Muscles of the oral region

Orbicularis oris forms the core of the muscles that move the mouth and lips. This muscle had distinct upper and lower parts, with some fibre bundles of the upper part overlapping the lower part at the angle of the mouth (Figure 5.7).

Figure 5.7. Three-dimensional model of orbicularis oris, anterior views. A. Lower part. B. Inferior fibre bundles of lower part. C. Deep fibre bundles of upper part. D. Superficial fibre bundles of upper part.

The lower part mainly consisted of long arched fibre bundles, but the most inferior fibre bundles were short and did not cross the midline. The upper part was divided into superficial and deep
fibre bundles. The deep fibre bundles were short and did not cross the midline, whereas the superficial fibre bundles were long and lay superficial to the fibre bundles of the lower part where they overlap at the angle of the mouth.

The fibre bundles of the other muscles of facial expression that move the mouth and lips, including buccinator, depressor anguli oris and levator anguli oris interdigitated with the fibre bundles of orbicularis oris (Figure 5.8). Buccinator is classically described as a “cheek” muscle, but was found to have extensive interdigitations with the lower part of orbicularis oris (Figure 5.8 A and B), with the upper part of orbicularis oris only interdigitating with a small slip of buccinator (Figure 5.8 C and D). The remainder of the upper part of orbicularis oris lay superficial to buccinator.

The fibre bundles of depressor anguli oris blended with the periosteum of the inferior border of the mandible and continued to course superiorly along the lateral side of the nasolabial fold to interdigitate with the upper part of orbicularis oris near the angle of the mouth (Figure 5.8 E and F). As the fibre bundles coursed superiority, they lay superficial to buccinator. At the angle of the mouth, the inferior part of levator anguli oris was interdigitated with depressor anguli oris, forming a continuous band of muscle (Figure 5.8. G and H).
Figure 5.8. Oral muscles surrounding the orbicularis oris (brown), anterior and lateral views. A and B. Buccinator (blue) and lower part of orbicularis oris. C and D. Addition of upper part of orbicularis oris. E and F. Addition of depressor anguli oris (magenta). G and H. Addition of levator anguli oris (green).
Zygomaticus major and minor did not interdigitate with orbicularis oris, but blended to orbicularis oris via connective tissue. This connective tissue spanned from the medial margin of zygomaticus major and the inferior fibre bundles of zygomaticus minor to the angle of the mouth. The superior fibre bundles of zygomaticus minor attached adjacent to the ala of the nose (Figure 5.9).

Figure 5.9. Three-dimensional model of zygomaticus major and minor in relation to orbicularis oris, anterior view.

The architectural parameters of the muscles of the oral region are summarized in Table 5.2.
Table 5.2. Architectural parameters of the muscles of the oral region

<table>
<thead>
<tr>
<th>Muscle</th>
<th>FBL (mm)</th>
<th>PA (°)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orbicularis oris</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Superior fibres of upper part</td>
<td>114.1 ± 9.8</td>
<td>X</td>
</tr>
<tr>
<td>Superior fibres of lower part</td>
<td>83.0 ± 17.3</td>
<td>X</td>
</tr>
<tr>
<td>Deep fibres of upper part (L)</td>
<td>35.5 ± 14.0</td>
<td>X</td>
</tr>
<tr>
<td>Deep fibres of upper part (R)</td>
<td>44.1 ± 6.2</td>
<td>X</td>
</tr>
<tr>
<td>Inferior fibres of lower part (L)</td>
<td>30.1 ± 4.3</td>
<td>X</td>
</tr>
<tr>
<td>Inferior fibres of lower part (R)</td>
<td>37.6 ± 10.7</td>
<td>X</td>
</tr>
<tr>
<td>Buccinator (R)</td>
<td>40.7 ± 13.5</td>
<td>26.9 ± 14.1</td>
</tr>
<tr>
<td>Depressor anguli oris (L)</td>
<td>40.6 ± 7.1</td>
<td>13.3 ± 8.0</td>
</tr>
<tr>
<td>Depressor anguli oris (R)</td>
<td>41.5 ± 13.1</td>
<td>19.8 ± 12.1</td>
</tr>
<tr>
<td>Levator anguli oris (L)</td>
<td>48.5 ± 10.2</td>
<td>13.0 ± 8.8</td>
</tr>
<tr>
<td>Levator anguli oris (R)</td>
<td>54.1 ± 16.1</td>
<td>23.4 ± 8.1</td>
</tr>
</tbody>
</table>

L, left; R, right; X, not quantified.

The mean FBL of the superficial upper and superior lower parts of orbicularis oris were longer than those of the deep upper and inferior lower parts, respectively (Table 5.2). Buccinator had a greater PA than both levator and depressor anguli oris. When comparing levator and depressor anguli oris, levator anguli oris was found to have a greater mean FBL. The right and left levator anguli oris and depressor anguli oris had similar PAs, with the right greater than the left.

5.2.1.2 Muscles of the nasal region

Nasalis, the deeper of two primary nasal muscles, lay on the lateral aspect of the bony and cartilaginous components of the nose (Figure 5.10). This muscle was found to be triangular in shape and had a small inferior attachment to the maxilla adjacent to the inferolateral part of the nasal notch. From its distal attachment, the fibre bundles diverged into three main parts attaching to connective tissue overlying the greater alar cartilage, lateral nasal cartilage and nasal bone (Figure 5.10 A).
Figure 5.10. Three-dimensional model of the muscles of the nasal region. A. Nasalis, anterior and lateral views. B. Alar part of levator labii superioris alaeque nasi (LLSAN), anterior and lateral views. C. Labial part of LLSAN, anterior and lateral views

Levator labii superioris alaeque nasi (LLSAN) was superficial to nasalis and was found to consist of two parts, alar and labial. Superiorly, both parts attached to the superior frontal process of the maxilla (Figure 5.10 B and C). Inferiorly, the alar part extended to the fibro-fatty tissue of the ala of the nose, and the labial part to the fascia of the superolateral portion of the upper lip.
Table 5.3. Architectural parameters of the muscles of the nasal region.

<table>
<thead>
<tr>
<th>Muscle</th>
<th>FBL (mm)</th>
<th>PA (°)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nasalis (L)</td>
<td>26.9 ± 4.8</td>
<td>41.4 ± 23.0</td>
</tr>
<tr>
<td>Nasalis (R)</td>
<td>36.6 ± 2.7</td>
<td>39.9 ± 25.0</td>
</tr>
<tr>
<td>Levator labii superioris alaeque nasi (L)</td>
<td>36.1 ± 7.6</td>
<td>13.5 ± 7.2</td>
</tr>
<tr>
<td>Alar part</td>
<td>31.2 ± 4.3</td>
<td>13.1 ± 4.8</td>
</tr>
<tr>
<td>Labial part</td>
<td>39.2 ± 7.6</td>
<td>13.8 ± 8.4</td>
</tr>
<tr>
<td>Levator labii superioris alaeque nasi (R)</td>
<td>30.8 ± 4.5</td>
<td>24.9 ± 7.3</td>
</tr>
<tr>
<td>Alar part</td>
<td>29.2 ± 3.8</td>
<td>25.9 ± 6.4</td>
</tr>
<tr>
<td>Labial part</td>
<td>32.9 ± 4.6</td>
<td>23.5 ± 8.3</td>
</tr>
</tbody>
</table>

L, left; R, right.

The FBL and PA of the two primary nasal muscles are summarized in Table 5.3. The right and left sides of each muscle were asymmetrical. The FBL of the right nasalis was 37% longer than that of the left, whereas the FBL of the left LLSAN was 20% longer than that of the right. In addition, the PA of the right and left LLSAN followed the same trend. Due to the divergent fibre bundle arrangement of nasalis, its PA was much greater than that of LLSAN. When the alar and labial parts of LLSAN were compared, the labial part of both sides was observed to have a longer FBL than the alar part, while the PAs were similar.

5.2.1.3 Muscles of the orbital region and scalp

Orbicularis oculi, corrugator supercillii, procerus and frontalis will be considered in this thesis.
The palpebral part of orbicularis oculi was the deepest and was overlapped by the circumferential orbital part (Figure 5.11 A and B). The small, superficial part of orbicularis oculi was located in the inferomedial part of the orbital region (Figure 5.11C). Its fibre bundles attached medially to the superior margin of the frontal process of the maxilla and the medial canthal ligament, and laterally the fibre bundles diverged to attach to the fascia of the infraorbital region. The superomedial fibre bundles of the orbital part overlapped the inferior fibre bundles of corrugator supercilii (Figure 5.11 D). Both corrugator supercilii and procerus were found to be fan-shaped muscles with their bases attaching inferiorly to the nasal process of the frontal bone and the nasal bone. Frontalis lay superficial to corrugator supercilii and interdigitated with the fibre bundles of procerus, attaching inferiorly to the supraorbital ridge deep to the orbital part of orbicularis oculi (Figure 5.11 E and F).
Table 5.4. Architectural parameters of the muscles of orbital region and scalp

<table>
<thead>
<tr>
<th>Muscle</th>
<th>FBL (mm)</th>
<th>PA (°)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orbicularis oculi (L)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Superficial</td>
<td>32.7 ± 7.2</td>
<td>X</td>
</tr>
<tr>
<td>Orbital part</td>
<td>112.0 ± 29.9</td>
<td>X</td>
</tr>
<tr>
<td>Palpebral part</td>
<td>54.2 ± 10.4</td>
<td>X</td>
</tr>
<tr>
<td>Orbicularis oculi (R)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Superficial</td>
<td>39.4 ± 9.6</td>
<td>X</td>
</tr>
<tr>
<td>Orbital part</td>
<td>86.9 ± 35.9</td>
<td>X</td>
</tr>
<tr>
<td>Palpebral part</td>
<td>65.6 ± 35.6</td>
<td>X</td>
</tr>
<tr>
<td>Frontalis (L)</td>
<td>73.2 ± 7.6</td>
<td>13.1 ± 8.1</td>
</tr>
<tr>
<td>Frontalis (R)</td>
<td>67.6 ± 7.1</td>
<td>15.6 ± 9.6</td>
</tr>
<tr>
<td>Corrugator supercili (L)</td>
<td>30.1 ± 5.7</td>
<td>9.1 ± 4.9</td>
</tr>
<tr>
<td>Corrugator supercili (R)</td>
<td>30.5 ± 5.1</td>
<td>10.0 ± 5.4</td>
</tr>
<tr>
<td>Procerus (L)</td>
<td>51.2 ± 6.7</td>
<td>22.3 ± 14.7</td>
</tr>
<tr>
<td>Procerus (R)</td>
<td>43.6 ± 10.2</td>
<td>18.1 ± 10.9</td>
</tr>
</tbody>
</table>

X, not quantified.

The architecture of orbicularis oculi is complex, with the orbital part having the longest mean FBL and the superficial part the shortest. In addition, due to its sphincter morphology, the orbital part of orbicularis oculi also had the longest FBL of all the muscles of the orbital and scalp regions. Corrugator supercili had on average shorter fibre bundles and a smaller PA than procerus (Table 5.4). The FBL of procerus was on average about 20mm shorter than that of frontalis. Asymmetry between the left and right sides was also found. For example, the mean FBL of the left orbital part of orbicularis oculi was about 30% longer than the right; however, both the superficial and palpebral parts of the right orbicularis oculi were found to be about 21% longer than their counterparts on the left.

5.2.2 Muscles of mastication

5.2.2.1 Temporalis

The temporalis muscle was divided into two distinct parts, superficial and deep, based on the areas of attachment and architecture (Figure 5.12).
The superficial part of temporalis attached proximally to the superficial surface of the temporal fossa spanning the frontal, parietal and temporal bones, and to the temporalis fascia. The deep part of temporalis attached to the temporal surface of the greater wing of the sphenoid bone, and the posterior surface of the zygomatic process of the frontal bone. Distally, the superficial and deep parts converged to attach to a thick, fan-shaped aponeurosis that continued to the medial surface and anterior border of the coronoid process of the mandible (Figure 5.13).
When considering the architecture of the superficial and deep parts of temporalis, the FBL of the superficial part was 24% longer than that of the deep part, and the MV of the superficial part was about 83% larger (Table 5.3).

<table>
<thead>
<tr>
<th>Temporalis</th>
<th>FBL (mm)</th>
<th>SL (μm)</th>
<th>PA (°)</th>
<th>PCSA (cm²)</th>
<th>MV (cm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole muscle</td>
<td>33.1 ± 7.74</td>
<td>1.6 ± 0.2</td>
<td>26.3 ± 15.5</td>
<td>7.7</td>
<td>30.6</td>
</tr>
<tr>
<td>Superficial part</td>
<td>35.6 ± 8.3</td>
<td>1.6 ± 0.2</td>
<td>27.5 ± 17.2</td>
<td>4.4</td>
<td>19.8</td>
</tr>
<tr>
<td>Deep part</td>
<td>28.7 ± 3.5</td>
<td>1.7 ± 0.1</td>
<td>24.3 ± 11.2</td>
<td>3.4</td>
<td>10.8</td>
</tr>
</tbody>
</table>

It is of interest to note that the MV of the superficial part of temporalis was approximately double that of the deep part, but the PCSA of the superficial part was only 23% greater than that of the deep part.

Based on computation of the principal fibre bundle orientations in the superficial and deep parts of temporalis, it was determined that the superficial part had four principal fibre
bundle orientations and the deep part had two (Figure 5.14). Principal fibre bundle orientation can be used to determine the direction of force generation (line of action) of each of the sub-volumes of a muscle in comparison to the line of action of the entire muscle.

Figure 5.14. Principal fibre bundle orientations (lines of action) of the superficial and deep parts of temporalis, lateral views. A. Superficial part. B. Deep part. Yellow arrows, principal fibre bundle orientations of the superficial and deep parts; black arrow, line of action of entire muscle.

As seen in Figure 5.14, the line of action of each of the sub-volumes changed direction from anterior to posterior. In the anterior sub-volumes of the superficial part of temporalis, the line of action was directed superiorly, whereas it was directed posteriorly in the posterior sub-volumes, providing evidence of functional differences between the sub-volumes. The line of action in the anterior sub-volume of the deep part of temporalis was directed superiorly, and that of the posterior sub-volume was directed superoposteriorly.

The sarcomere length of temporalis was measured from 13 locations throughout the muscle volume. The mean sarcomere length of the superficial and deep parts of temporalis was found to be similar: $1.6 \pm 0.2\mu m$ and $1.7 \pm 0.1\mu m$, respectively (Table 5.5). However, when
sarcomere length was graphed to the location of the biopsy sites from anterior to posterior, the posteriorly located muscle fibres tended to have a shorter sarcomere length than the more anterior fibres (Figure 5.15).

![Sarcomere length variation within the volume of temporalis.](image)

**Figure 5.15.** Sarcomere length variation within the volume of temporalis.

### 5.2.2.2 Masseter

Masseter was found to be a quadrangular muscle located on the lateral surface of the mandible (Figure 5.16). It consisted of superficial and deep heads that were further subdivided into laminae.
The left and right masseter muscles were each found to contain 8 laminae numbered from superficial to deep (Figure 5.17). The superficial head consisted of the first four laminae, and the deep head consisted of the last four laminae.
Figure 5.17. Laminae of masseter. A. Laminae (1-4) of the superficial head. B. Laminae (5-8) of the deep head.

The fibre bundles of each of the laminae attached to alternating sheets of superior and inferior aponeuroses or directly to bone. There were four superior aponeuroses and four to five inferior aponeuroses. The locations of fibre bundle attachments in each of the laminae are summarized in Table 5.6 and also visually represented in Figures 5.18 and 19.
Table 5.6. Summary of fibre bundle attachment sites in each lamina (1-8) of masseter.

<table>
<thead>
<tr>
<th>Lamina</th>
<th>Superior attachment</th>
<th>Inferior attachment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>Superficial head</strong></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Via aponeurosis attaching to zygomatic process of maxilla (Figure 5.18 A)</td>
<td>Bone: inferior border of ramus of mandible (Figure 5.18 C)</td>
</tr>
<tr>
<td>2</td>
<td>Via aponeurosis attaching to zygomatic process of maxilla (Figure 5.18 A)</td>
<td>Via aponeurosis attaching to angle and inferior border of ramus of mandible (Figure 5.18 C)</td>
</tr>
<tr>
<td>3</td>
<td>Via aponeurosis attaching to anterior 1/3 of zygomatic arch (Figure 5.18 B)</td>
<td>Attach to angle and inferior border of ramus of mandible directly or via aponeurosis (Figure 5.18 C).</td>
</tr>
<tr>
<td>4</td>
<td>Via aponeurosis attaching to middle 1/3 of zygomatic arch (Figure 5.18 D)</td>
<td>Attach to posterior border of ramus of mandible directly or via aponeurosis (Figure 5.18 E)</td>
</tr>
<tr>
<td></td>
<td><strong>Deep head</strong></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Superficial surface of deep aponeurosis attaching to middle 1/3 of zygomatic arch (Figure 5.19 A)</td>
<td>Via aponeurosis attaching to ramus of mandible centrally (Figure 5.19 B)</td>
</tr>
<tr>
<td>6</td>
<td>Superficial surface of deep aponeurosis attaching to posterior 1/3 of zygomatic arch (Figure 5.19 A)</td>
<td>Via aponeurosis attaching to superior part of ramus of mandible (Figure 5.19 C)</td>
</tr>
<tr>
<td>7</td>
<td>Deep surface of deep aponeurosis attaching to posterior 2/3 of zygomatic arch (Figure 5.19 A)</td>
<td>Via aponeurosis attaching to coronoid process of mandible (Figure 5.19 C)</td>
</tr>
<tr>
<td>8</td>
<td>Bone: mandibular notch</td>
<td>Bone: posterior surface of zygomatic notch</td>
</tr>
</tbody>
</table>
Figure 5.18. Aponeurotic attachment sites of laminae (1-4) of the superficial head of masseter. A. Superior attachments of laminae 1 and 2. B. Superior attachment of lamina 3. C. Superior attachment of lamina 4. D. Inferior attachments of laminae 1, 2 and 3. E. Inferior attachment of lamina 4. Blue, must superficial aponeurosis of each of the images. Dotted outline
Figure 5.19. Aponeurotic attachment sites of laminae (5-8) of the deep head of masseter. A. Superior attachments of laminae 5, 6 and 7. B. Inferior attachment of lamina 5. C. Inferior attachments of laminae 6 and 7.

The architectural parameters of masseter were quantified for the muscle as a whole, the superficial and deep heads, and individual laminae (Figure 5.20 and Table 5.7).

Figure 5.20. Architectural parameters of the superficial and deep heads of masseter. The mean FBL and PA and total PCSA and MV of the superficial and deep heads are indicated above each bar.
When the architectural parameters of the superficial and deep heads were compared, the mean FBL of the superficial head was found to be 31% greater than that of the deep head. In contrast, the mean PA of the deep head was 58% greater than that of the superficial head. In terms of size, the superficial head was greater than the deep head in both PCSA and volume, constituting 63% of the total PCSA and 68% of the total MV of the entire muscle (Figure 5.20 and Table 5.7).

<table>
<thead>
<tr>
<th>Masseter</th>
<th>FBL (mm)</th>
<th>SL (μm)</th>
<th>PA (˚)</th>
<th>PCSA (cm²)</th>
<th>MV (cm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Masseter right</td>
<td>28.3 ± 6.5</td>
<td>1.8 ± 0.2</td>
<td>17.2 ± 11.0</td>
<td>9.8</td>
<td>28.7</td>
</tr>
<tr>
<td>Superficial head</td>
<td>30.5 ± 4.8</td>
<td>1.8 ± 0.1</td>
<td>15.4 ± 9.2</td>
<td>6.4</td>
<td>20.5</td>
</tr>
<tr>
<td>Lamina 1</td>
<td>33.2 ± 3.2</td>
<td>1.8 ± 0.2</td>
<td>24.8 ± 9.3</td>
<td>1.4</td>
<td>5.1</td>
</tr>
<tr>
<td>Lamina 2</td>
<td>30.9 ± 3.6</td>
<td>1.8 ± 0.1</td>
<td>17.5 ± 7.1</td>
<td>1.5</td>
<td>4.7</td>
</tr>
<tr>
<td>Lamina 3</td>
<td>30.1 ± 6.0</td>
<td>1.8 ± 0.1</td>
<td>10.5 ± 5.1</td>
<td>1.7</td>
<td>5.4</td>
</tr>
<tr>
<td>Lamina 4</td>
<td>28.7 ± 4.5</td>
<td>1.8 ± 0.1</td>
<td>8.1 ± 3.8</td>
<td>1.2</td>
<td>3.4</td>
</tr>
<tr>
<td>Deep head</td>
<td>22.2 ± 6.7</td>
<td>1.5 ± 0.2</td>
<td>23.8 ± 12.9</td>
<td>3.4</td>
<td>8.2</td>
</tr>
<tr>
<td>Lamina 5</td>
<td>28.0 ± 4.4</td>
<td>1.8 ± 0.0</td>
<td>7.8 ± 3.5</td>
<td>0.7</td>
<td>1.9</td>
</tr>
<tr>
<td>Lamina 6</td>
<td>21.4 ± 6.3</td>
<td>X</td>
<td>22.2 ± 9.5</td>
<td>1.7</td>
<td>4.0</td>
</tr>
<tr>
<td>Lamina 7</td>
<td>24.7 ± 6.9</td>
<td>1.3 ± 0.1</td>
<td>22.6 ± 13.7</td>
<td>1.3</td>
<td>3.6</td>
</tr>
<tr>
<td>Lamina 8</td>
<td>16.1 ± 2.6</td>
<td>X</td>
<td>38.3 ± 17.1</td>
<td>0.3</td>
<td>0.7</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Masseter</th>
<th>FBL (mm)</th>
<th>SL (μm)</th>
<th>PA (˚)</th>
<th>PCSA (cm²)</th>
<th>MV (cm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Masseter left</td>
<td>25.9 ± 5.2</td>
<td>1.9 ± 0.2</td>
<td>16.7 ± 10.8</td>
<td>8.7</td>
<td>23.8</td>
</tr>
<tr>
<td>Superficial head</td>
<td>27.8 ± 4.4</td>
<td>2.0 ± 0.2</td>
<td>13.8 ± 7.3</td>
<td>5.2</td>
<td>15.0</td>
</tr>
<tr>
<td>Lamina 1</td>
<td>28.9 ± 3.7</td>
<td>1.8 ± 0.1</td>
<td>16.6 ± 7.7</td>
<td>1.7</td>
<td>5.2</td>
</tr>
<tr>
<td>Lamina 2</td>
<td>31.9 ± 5.4</td>
<td>2.0 ± 0.0</td>
<td>9.6 ± 5.5</td>
<td>0.6</td>
<td>1.9</td>
</tr>
<tr>
<td>Lamina 3</td>
<td>25.9 ± 2.8</td>
<td>2.0 ± 0.1</td>
<td>12.1 ± 7.3</td>
<td>1.3</td>
<td>3.6</td>
</tr>
<tr>
<td>Lamina 4</td>
<td>26.4 ± 4.3</td>
<td>2.1 ± 0.1</td>
<td>13.3 ± 5.9</td>
<td>1.6</td>
<td>4.4</td>
</tr>
<tr>
<td>Deep head</td>
<td>22.3 ± 4.6</td>
<td>1.6 ± 0.2</td>
<td>22.3 ± 13.7</td>
<td>3.6</td>
<td>8.8</td>
</tr>
<tr>
<td>Lamina 5</td>
<td>21.6 ± 2.8</td>
<td>1.8 ± 0.1</td>
<td>15.7 ± 8.0</td>
<td>1.2</td>
<td>2.8</td>
</tr>
<tr>
<td>Lamina 6</td>
<td>23.1 ± 6.5</td>
<td>X</td>
<td>19.6 ± 9.6</td>
<td>1.2</td>
<td>3.0</td>
</tr>
<tr>
<td>Lamina 7</td>
<td>22.2 ± 2.5</td>
<td>1.6 ± 0.1</td>
<td>26.9 ± 15.0</td>
<td>0.8</td>
<td>2.1</td>
</tr>
<tr>
<td>Lamina 8</td>
<td>21.4 ± 3.0</td>
<td>1.3 ± 0.0</td>
<td>46.3 ± 11.9</td>
<td>0.3</td>
<td>0.9</td>
</tr>
</tbody>
</table>

X, not quantified.

The FBL varied through the lamina, ranging from 33.2 ± 3.2mm superficially to 16.1 ± 2.6mm deeply in the right masseter and 31.9 ± 5.4mm to 21.4 ± 3.0mm in the left masseter.
(Table 5.7). The longest average FBL was found in laminae 1 and 2, and the shortest in lamina 8 (Figure 5.21).

![Fibre bundle length (mm)](image1)

**Figure 5.21. Mean FBL of individual laminae of right masseter.**

The PA was found to decrease from lamina 1 to 4 in the superficial head, and then increase from lamina 5 to 8 in the deep head (Figure 5.22 and Table 5.7). Laminae 3 and 4 had the smallest PA and lamina 8 had the largest.

![Pennation angle (°)](image2)

**Figure 5.22. Mean PA of individual laminae of right masseter.**
No pattern was observed for individual laminae when considering PCSA and MV, but as mentioned above, at the level of the superficial and deep heads, considerable differences were noted, with the superficial head having a greater MV and PCSA than the deep head.

The principal fibre bundle orientations (lines of action) differed in the anterior and posterior parts of the superficial and deep heads of masseter (Figure 5.23). The line of action of the anterior part of the superficial head was directed anterosuperiorly in the sagittal plane and superolaterally in the coronal plane; that of the posterior part of the superficial head was directed superiorly in the sagittal plane and superolaterally in the coronal plane; and in the deep head, superoposteriorly and superolaterally, respectively.

Figure 5.23. Principal fibre bundle orientations (lines of action) of the superficial and deep heads of masseter. A. Lateral view. B. Anterior view. Red fibre bundles, anterior part of superficial head; blue fibre bundles, posterior part of superficial head; green fibre bundles, deep head; yellow arrows, principal fibre bundle orientations of the superficial and deep heads; black arrow, line of action of entire muscle.
The SL was measured at 12 biopsy sites. The SL of the superficial head (right: 1.8 ± 0.1μm; left: 2.0 ± 0.2μm) was found to be longer than that of the deep head (right: 1.5 ± 0.2μm; left: 1.6 ± 0.2μm). In addition, in both the right and left masseter muscles, the SL of lamina 8 (right: 1.3 ± 0.0; left: 1.3 ± 0.1μm) was found to be much shorter than the overall average for the entire muscle (right: 1.8μm; left: 1.9μm).

5.2.2.3 Medial pterygoid

The medial pterygoid was found to have anterior and posterior parts. The anterior region attached to the pyramidal process of the palatine bone and the maxillary tuberosity, whereas the posterior region attached to the medial surface of the lateral pterygoid plate (Figure 5.24).

The anterior part of the medial pterygoid was muscular with fibre bundles arranged in parallel (Figure 5.25 A). In contrast, the posterior parts had a large number of aponeuroses spanned by fibre bundles, making this part of the muscle multipennate and architecturally complex (Figure 5.25. B to D).
Figure 5.25. Three-dimensional model of the medial pterygoid. A. Anterior (green) and posterior (red) regions, lateral view. B. Posterior region, lateral view. C. Internal aponeuroses, lateral view. D. Internal aponeuroses, anterior view. LP, lateral pterygoid plate.

The posterior part was larger than the anterior part, but their FBL, SL and PA were similar (Table 5.8). Interestingly, the MV of the posterior part was found to be 55% larger than that of the anterior part, but the PCSA of the posterior part was 78% larger than that of the anterior part.

Table 5.8. Architectural parameters of the medial pterygoid.

<table>
<thead>
<tr>
<th>Medial pterygoid</th>
<th>FBL (mm)</th>
<th>SL (μm)</th>
<th>PA (°)</th>
<th>PCSA (cm²)</th>
<th>MV (cm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole muscle</td>
<td>20.5 ± 4.5</td>
<td>2.1 ± 0.2</td>
<td>11.1 ± 6.4</td>
<td>5.0</td>
<td>10.6</td>
</tr>
<tr>
<td>Anterior part</td>
<td>21.6 ± 3.6</td>
<td>2.0 ± 0.1</td>
<td>12.6 ± 6.9</td>
<td>1.8</td>
<td>4.2</td>
</tr>
<tr>
<td>Posterior part</td>
<td>20.0 ± 4.8</td>
<td>2.1 ± 0.2</td>
<td>10.3 ± 6.1</td>
<td>3.2</td>
<td>6.5</td>
</tr>
</tbody>
</table>

In addition, the principal orientations of the fibre bundles of the anterior and posterior parts, i.e., lines of action, were relatively similar. Both were oriented superiorly in the sagittal plane and superomedially in the coronal plane (Figure 5.26).
5.2.2.4 Lateral pterygoid

The lateral pterygoid was divided into superior and inferior heads based on its attachment sites, as well as its fibre bundle orientations (Figure 5.27). Proximally, the superior head attached to the infratemporal crest of the greater wing of the sphenoid bone and the inferior head attached to the lateral surface of the lateral pterygoid plate. Distally, both the superior and inferior heads attached to the medial part of the head (condyloid process) of the mandible. In addition, some fibre bundles of the superior head inserted onto the capsule of the temporomandibular joint.
The superior and inferior heads had relatively uniform architecture, as FBL, PA and SL were comparable (Table 5.9). The inferior head was almost twice the size of the superior head; this was reflected in the MV and PCSA.

Table 5.9. Architectural parameters of the lateral pterygoid.

<table>
<thead>
<tr>
<th>Lateral pterygoid</th>
<th>FBL (mm)</th>
<th>SL (μm)</th>
<th>PA (˚)</th>
<th>PCSA (cm²)</th>
<th>MV (cm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole muscle</td>
<td>28.0 ± 4.0</td>
<td>1.9 ± 0.2</td>
<td>12.1 ± 6.6</td>
<td>3.2</td>
<td>8.9</td>
</tr>
<tr>
<td>Superior head</td>
<td>27.8 ± 5.0</td>
<td>1.9 ± 0.1</td>
<td>11.1 ± 4.8</td>
<td>1.1</td>
<td>3.0</td>
</tr>
<tr>
<td>Inferior head</td>
<td>28.0 ± 3.5</td>
<td>1.9 ± 0.2</td>
<td>12.5 ± 7.2</td>
<td>2.1</td>
<td>5.9</td>
</tr>
</tbody>
</table>

The principal fibre bundle orientation of the superior head was horizontal and directed medially in the coronal plane and slightly anteromedially in the sagittal plane. In contrast, the principal fibre bundle orientation of the inferior head was directed inferomedially in the coronal plane and slightly inferoanteriorly in the sagittal plane (Figure 5.28).
Figure 5.28. Principal fibre bundle orientations (lines of action) of the superior and inferior heads of the lateral pterygoid. A. Lateral view. B. Anterior view. Red fibre bundles, superior head; yellow fibre bundles, inferior head; yellow arrows, principal fibre bundle orientations of the superior and inferior heads; black arrow, line of action of entire muscle.

5.2.2.5 Architectural summary of muscles of mastication

The architectural parameters of each of the muscles of mastication and their component parts are summarized in Table 5.10 and compared below.

Table 5.10. Summary of mean architectural parameters of muscles of mastication.

<table>
<thead>
<tr>
<th>Muscle</th>
<th>FBL (mm)</th>
<th>SL (μm)</th>
<th>PA (˚)</th>
<th>PCSA (cm²)</th>
<th>MV (cm³)</th>
<th>Force index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temporals</td>
<td>33.1 ± 7.74</td>
<td>1.6 ± 0.2</td>
<td>26.3 ± 15.5</td>
<td>7.7</td>
<td>30.6</td>
<td>0.79</td>
</tr>
<tr>
<td>Superficial</td>
<td>35.6 ± 8.3</td>
<td>1.6 ± 0.2</td>
<td>27.5 ± 17.2</td>
<td>4.4</td>
<td>19.8</td>
<td>0.60</td>
</tr>
<tr>
<td>Deep</td>
<td>28.7 ± 3.5</td>
<td>1.7 ± 0.1</td>
<td>24.3 ± 11.2</td>
<td>3.4</td>
<td>10.8</td>
<td>0.70</td>
</tr>
<tr>
<td>Masseter right</td>
<td>28.3 ± 6.5</td>
<td>1.8 ± 0.2</td>
<td>17.2 ± 11.0</td>
<td>9.8</td>
<td>28.7</td>
<td>1.05</td>
</tr>
<tr>
<td>Superficial</td>
<td>30.5 ± 4.8</td>
<td>1.8 ± 0.1</td>
<td>15.4 ± 9.2</td>
<td>6.4</td>
<td>20.5</td>
<td>0.85</td>
</tr>
<tr>
<td>Deep</td>
<td>22.2 ± 6.7</td>
<td>1.5 ± 0.2</td>
<td>23.8 ± 12.9</td>
<td>3.4</td>
<td>8.2</td>
<td>0.84</td>
</tr>
<tr>
<td>Masseter left</td>
<td>25.9 ± 5.2</td>
<td>1.9 ± 0.2</td>
<td>16.7 ± 10.8</td>
<td>8.7</td>
<td>23.8</td>
<td>1.05</td>
</tr>
<tr>
<td>Superficial</td>
<td>27.8 ± 4.4</td>
<td>2.0 ± 0.2</td>
<td>13.8 ± 7.3</td>
<td>5.2</td>
<td>15.0</td>
<td>0.85</td>
</tr>
<tr>
<td>Deep</td>
<td>22.3 ± 4.6</td>
<td>1.6 ± 0.2</td>
<td>22.3 ± 13.7</td>
<td>3.6</td>
<td>8.8</td>
<td>0.84</td>
</tr>
<tr>
<td>Medial pterygoid</td>
<td>20.5 ± 4.5</td>
<td>2.1 ± 0.2</td>
<td>10.0 ± 5.9</td>
<td>4.9</td>
<td>10.7</td>
<td>1.04</td>
</tr>
<tr>
<td>Anterior</td>
<td>21.6 ± 3.6</td>
<td>2.0 ± 0.1</td>
<td>12.6 ± 6.9</td>
<td>1.8</td>
<td>4.2</td>
<td>0.69</td>
</tr>
<tr>
<td>Posterior</td>
<td>20.0 ± 4.8</td>
<td>2.1 ± 0.2</td>
<td>10.3 ± 6.1</td>
<td>3.2</td>
<td>6.5</td>
<td>0.92</td>
</tr>
<tr>
<td>Lateral pterygoid</td>
<td>28.0 ± 4.0</td>
<td>1.9 ± 0.2</td>
<td>12.1 ± 6.6</td>
<td>3.2</td>
<td>8.9</td>
<td>0.75</td>
</tr>
<tr>
<td>Superior</td>
<td>27.8 ± 5.0</td>
<td>1.9 ± 0.1</td>
<td>11.1 ± 4.8</td>
<td>1.1</td>
<td>3.0</td>
<td>0.53</td>
</tr>
<tr>
<td>Inferior</td>
<td>28.0 ± 3.5</td>
<td>1.9 ± 0.2</td>
<td>12.5 ± 7.2</td>
<td>2.1</td>
<td>5.9</td>
<td>0.64</td>
</tr>
</tbody>
</table>
When the FBLs of all of the muscles of mastication were compared, the temporalis was found to have the longest FBL (33.1 ± 7.74mm), and the medial pterygoid the shortest (20.5 ± 4.5mm). Fibre bundle length decreased from the superficial to deep parts/heads of temporalis and masseter, e.g., the deep head of masseter was on average 5-8mm shorter than the superficial head. The medial and lateral pterygoids had a consistent FBL throughout their volumes. Sarcomere length was generally found to range from 1.8 to 2.1 μm, except in temporalis and the deep head of masseter, which were found to have the shortest SL, ranging from 1.5 to 1.7 μm.

Pennation angle varied between the muscles, with the medial pterygoid having the smallest mean PA (10.0 ± 5.9°), and temporalis the largest (26.3 ± 15.5°). The deep part of temporalis had a smaller mean PA than the superficial part, but interestingly, in masseter, the PA increased almost 10° from superficial to deep. The medial and lateral pterygoids had the smallest mean PAs, which were consistent throughout each part of the muscle.

The force index, which is a normalization of PCSA to MV, quantifies a muscle’s relative force-generating capabilities. The left and right masseters and the medial pterygoid were found to have the largest force indices (1.05 and 1.04, respectively). The medial pterygoid, although a small muscle by volume, had a large force index due to the high ratio between its PCSA and MV. In contrast, temporalis and the lateral pterygoid had much smaller force indices (0.79 and 0.75, respectively), suggesting a tendency towards excursion. Although temporalis had the largest MV and a relatively large PCSA compared to the other muscles of mastication, the ratio between its PCSA and MV was smaller, thus yielding a smaller force index. Despite the differences between the MVs of the left and right masseters, their force indices were the same.

Similarities in the principal fibre bundle orientations of the parts of the muscles of mastication were identified, providing evidence for potential functional synergies. For example, the deep head of the masseter and the anterior sub-volumes of the superficial and deep parts of
temporals were observed to have similar principal fibre bundle orientations, which were directed superiorly with a slight posterior deviation (Figure 5.29 A to C). In addition, the superficial head of masseter and the medial pterygoid had similar principal fibre bundle orientations in the sagittal plane. However, in the coronal plane, the fibre bundles of the superficial head of masseter were directed superolaterally, whereas in the medial pterygoid, they were directed superomedially (Figure 5.29 D to G).

Figure 5.29. Similarities in principal fibre bundle orientations of the parts of the muscles of mastication, lateral and anterior views. A to C. Superficial and deep parts of temporals and deep head of masseter, later view. AST, anterior sub-volumes of superficial part of temporals. D to G. Superficial head of masseter and the medial pterygoid. Yellow arrows, principal fibre bundle orientations of the parts of the muscles.
5.2.3 Suprahyoid muscles

5.2.3.1 Digastric

The digastric muscle was found to consist of two bellies connected by a cord-like intermediate tendon, which attached to the hyoid bone via the trochlea, a dense connective tissue sling (Figure 5.30). The anterior belly attached to the mandible at the digastric fossa, and the posterior belly attached to the medial surface of the mastoid process.

Figure 5.30. Three-dimensional model of digastric. A. Right and left digastric, inferior view. B. Right digastric, lateral view. C. Left digastric, lateral view. Light green fibre bundles, anterior belly of digastric; dark green fibre bundles, posterior belly of digastric. H, hyoid bone; T, trochlea.

The mean FBLs of the left and right anterior bellies of digastric were similar, but the
posterior bellies of digastric were asymmetrical, with the right side having a mean FBL 7mm longer than the left. The SL of the posterior belly was found to be 0.3 to 0.5μm longer than that of the anterior belly. Also, the posterior belly had a larger PA than the anterior belly (Table 5.11). On average, the MV of the posterior belly was about 15% larger than that of the anterior belly on both the left and right sides. Interestingly, on the right, the PCSA of the anterior belly was found to be 10% larger than that of the posterior belly, whereas on the left, the reverse was observed, i.e., the PCSA of the posterior belly was 10% larger than that of the anterior belly.

Table 5.11. Architectural parameters of digastric.

<table>
<thead>
<tr>
<th></th>
<th>FBL (mm)</th>
<th>SL (μm)</th>
<th>PA (°)</th>
<th>PCSA (cm²)</th>
<th>MV (cm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Right</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anterior belly</td>
<td>25.5 ± 4.1</td>
<td>1.4 ± 0.1</td>
<td>10.7 ± 5.3</td>
<td>0.95</td>
<td>2.5</td>
</tr>
<tr>
<td>Posterior belly</td>
<td>33.1 ± 6.3</td>
<td>1.7 ± 0.1</td>
<td>12.0 ± 5.8</td>
<td>0.86</td>
<td>2.9</td>
</tr>
<tr>
<td><strong>Left</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anterior belly</td>
<td>25.4 ± 4.1</td>
<td>1.5 ± 0.1</td>
<td>10.7 ± 6.5</td>
<td>0.96</td>
<td>2.5</td>
</tr>
<tr>
<td>Posterior belly</td>
<td>26.2 ± 6.2</td>
<td>2.0 ± 0.2</td>
<td>12.9 ± 7.4</td>
<td>1.06</td>
<td>3.0</td>
</tr>
</tbody>
</table>

Depending on the effector (the mandible or the hyoid bone), the direction of the combined line of action of the anterior and posterior bellies of digastric differ. When the effector is the mandible, the resultant line of action of the entire digastric is directed posterosuperiorly (Figure 5.31 A). In contrast, when the effector is the hyoid, the line of action of the anterior digastric is directed anteriorly, while that of the posterior digastric is directed posterosuperiorly, producing a resultant line of action for the entire digastric muscle that was directed anterosuperiorly (Figure 5.31 B).
Figure 5.31. Principal fibre bundle orientations (lines of action) of the anterior and posterior bellies of digastric, lateral views. A. Resultant mandibular movement. B. Resultant hyoid movement. Light green fibre bundles, anterior belly of digastric; dark green fibre bundles, posterior belly of digastric; yellow arrows, principal line of action of each belly; black arrow, resultant line of action of entire muscle.

5.2.3.2 Stylohyoid

Stylohyoid was found to be a slender muscle attached superiorly to the styloid process of the temporal bone and inferiorly to the greater horn of the hyoid bone via a broad tendon (Figure 5.32). The intermediate tendon of the digastric muscle pierced the belly of stylohyoid and divided the muscle into superficial and deep parts. Where the tendon of digastric traversed stylohyoid, it was loosely stabilized by surrounding connective tissue.
Figure 5.32. Three-dimensional model of stylohyoid, lateral view. Purple fibre bundles, superficial part; blue fibre bundles, deep part. IT, intermediate tendon of digastric; SP, styloid process.

Fibre bundle length was on average 5mm longer in the deep part of stylohyoid than in the superficial part, while SL was about 1.5 times longer in the deep part than in the superficial part (Table 5.12). The mean PA of the superficial part was almost twice that of the deep part. The superficial part constituted only 19% of the total MV and 21% of the total PCSA of the entire muscle, while the remaining 80% belonged to the deep part.

Table 5.12. Architectural parameters of stylohyoid and its parts.

<table>
<thead>
<tr>
<th>Stylohyoid</th>
<th>FBL (mm)</th>
<th>SL (μm)</th>
<th>PA (˚)</th>
<th>PCSA (cm²)</th>
<th>MV (cm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole muscle</td>
<td>46.7 ± 5.5</td>
<td>2.1 ± 0.4</td>
<td>11.0 ± 6.0</td>
<td>0.34</td>
<td>1.6</td>
</tr>
<tr>
<td>Superficial part</td>
<td>43.6 ± 1.6</td>
<td>1.5 ± 0.1</td>
<td>16.2 ± 4.9</td>
<td>0.07</td>
<td>0.3</td>
</tr>
<tr>
<td>Deep part</td>
<td>48.1 ± 6.0</td>
<td>2.3 ± 0.2</td>
<td>8.8 ± 5.1</td>
<td>0.27</td>
<td>1.3</td>
</tr>
</tbody>
</table>

The principal fibre bundle orientations of the superficial and deep parts of stylohyoid were similar and were directed superoposteriorly (Figure 5.33 A). Due to displacement by the
intermediate tendon of digastric, the principal fibre bundle orientation of the superficial part had a slight lateral deviation in the coronal plane (Figure 5.33 B).

![Figure 5.33. Principal fibre bundle orientations (lines of action) of the superficial and deep parts of stylohyoid. Purple fibre bundles, superficial part; blue fibre bundles, deep part. A. Lateral view. B. Anterior view. Yellow arrows, principal fibre bundle orientations of the superficial and deep parts; black arrow, line of action of entire muscle.](image)

5.2.3.3 Mylohyoid

Mylohyoid was found to be a broad suspensory muscle that formed the floor of the mouth (Figure 5.34). It was divided into three parts: anterior, middle and posterior (Figure 5.34 C and D). The anterior part was triangular and its fibre bundles spanned across the floor of the mouth, bridging the left and right sides of the mandible. In contrast, both the middle and posterior parts were split into right and left halves. The fibre bundles of the middle part attached to a raphe and those of the posterior part to an aponeurosis attaching to the hyoid bone (Figure 5.34 B and C). In addition, the middle part was perforated by the sublingual glandular tissue (sublingual boutonniere), which separated the muscle fibre bundles. Laterally, all three parts attached to the mylohyoid line of the mandible, extending from the mandibular symphysis anteriorly to the ramus of the mandible posteriorly.
The mylohyoid muscle is architecturally heterogeneous (Table 5.13). The posterior part was found to have the largest mean FBL (51.0 ± 4.8mm). The mean FBLs of the anterior and middle parts were similar (44.9 ± 14.0mm and 43.7 ± 8.0mm, respectively). However, due to the triangular shape of the anterior part, the FBL ranged from 10.4mm at the apex to 69.1mm at the base. In addition, the FBL of the right half of both the middle and posterior parts was found to be 13% and 17% longer, respectively, than the FBL of the left side.
The anterior and middle parts had the largest PA. In the middle part, the mean PA on the left side was found to be 12° or 20% greater than on the right. Similarly, in the posterior part, the mean PA on the left side was 30° or 68% greater than on the right.

Table 5.13. Architectural parameters of mylohyoid and its parts

<table>
<thead>
<tr>
<th>Mylohyoid</th>
<th>FBL (mm)</th>
<th>SL (μm)</th>
<th>PA</th>
<th>PCSA (cm$^2$)</th>
<th>MV (cm$^3$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole muscle</td>
<td>46.6 ± 9.7</td>
<td>2.0 ± 0.2</td>
<td>63.1 ± 10.9</td>
<td>1.40</td>
<td>12.4</td>
</tr>
<tr>
<td>Anterior part</td>
<td>44.9 ± 14.0</td>
<td>1.8 ± 0.2</td>
<td>65.1 ± 5.6</td>
<td>0.40</td>
<td>2.1</td>
</tr>
<tr>
<td>Middle* part</td>
<td>43.7 ± 8.0</td>
<td>2.0 ± 0.2</td>
<td>66.1 ± 6.6</td>
<td>0.46</td>
<td>5.0</td>
</tr>
<tr>
<td>Left</td>
<td>40.9 ± 8.1</td>
<td>1.9 ± 0.2</td>
<td>72.3 ± 6.6</td>
<td>0.18</td>
<td>2.5</td>
</tr>
<tr>
<td>Right</td>
<td>46.2 ± 7.0</td>
<td>2.0 ± 0.1</td>
<td>60.5 ± 3.1</td>
<td>0.28</td>
<td>2.6</td>
</tr>
<tr>
<td>Posterior* part</td>
<td>51.0 ± 4.8</td>
<td>2.1 ± 0.1</td>
<td>56.7 ± 16.6</td>
<td>0.54</td>
<td>5.3</td>
</tr>
<tr>
<td>Left</td>
<td>46.5 ± 2.5</td>
<td>2.1 ± 0.2</td>
<td>74.4 ± 8.0</td>
<td>0.15</td>
<td>2.3</td>
</tr>
<tr>
<td>Right</td>
<td>54.3 ± 3.1</td>
<td>2.1 ± 0.1</td>
<td>44.0 ± 6.5</td>
<td>0.39</td>
<td>3.0</td>
</tr>
</tbody>
</table>

* Raphe was not at midline.

The MV of the middle and posterior parts of mylohyoid was similar and each constituted 40% of the total volume, whereas the anterior part was smaller and comprised the remaining 20%. The posterior part was found to have the greatest PCSA, while the anterior part had the smallest. Although the MV of the middle part was two times greater than that of the anterior part, the PCSA of the posterior part was only 15% larger than that of the anterior part.

Due to its suspensory type of morphology, the lines of action of the entire mylohyoid muscle and its individual parts were determined by taking the average fibre bundle orientation of the left and right halves at the medial attachments of the fibre bundles (Figure 5.35, also see Section 4.5.2). As the fibre bundles of the anterior part of the mylohyoid spanned across the entire floor of the mouth, the principal fibre bundle orientation was computed at the midpoint of the fibre bundles (Figure 5.35 B).
Figure 5.35. Principal fibre bundle orientations and resultant lines of action of the individual parts of mylohyoid. A. Principal fibre bundle orientation and resultant line of action of anterior part, anterior view. B. Principal fibre bundle orientation and resultant line of action of middle part, anterior view. C. Principal fibre bundle orientation and resultant line of action of posterior part, anterior view. D. Lines of action of mylohyoid and its parts, lateral view, left half only. Red fibre bundles, anterior part; green fibre bundles, middle part; blue fibre bundles, posterior part; yellow arrows, principal fibre bundle orientations of the individual parts; orange arrow, resultant line of action of each part; black arrow, line of action of entire muscle.

The line of action was computed for the individual parts of mylohyoid and then used to infer their actions on the floor of the mouth and the hyoid bone. The line of action of the anterior part was directed superiorly in both the coronal and sagittal planes, suggesting that it may play a
role in elevation of the floor of the mouth (Figure 5.35 A and D). The line of action of the middle part was directed superiorly in the coronal plane and superoanteriorly in sagittal plane. As the fibre bundles of the middle part attached at the midline onto the raphe, and indirectly via the raphe to the hyoid, this suggests that the middle part may have a role in elevating the floor of the mouth, as well as elevating and anteriorly displacing the hyoid. The line of action of the posterior part was directed superiorly in both the coronal and sagittal planes, suggesting that it may contribute to direct elevation of the hyoid.

5.2.3.4 Geniohyoid

The right and left geniohyoid muscles were found to be separated by a thin fascial layer at the midline (Figure 5.36). Anteriorly, the geniohyoid attached to the inferior mental spine of the mandible, and coursed posteriorly attached to the body of hyoid.

![Figure 5.36. Three-dimensional model of geniohyoid. A. Inferior view. B. Lateral view. Green fibre bundles, right geniohyoid; yellow fibre bundles, left geniohyoid. H, hyoid bone.](image)

Fibre bundle length, SL and PA were found to be similar between the right and left sides. However, the PCSA and MV of the left side were about 20% larger than those of the right side.
Table 5.14. Architectural parameters of geniohyoid

<table>
<thead>
<tr>
<th>Geniohyoid</th>
<th>FBL (mm)</th>
<th>SL (μm)</th>
<th>PA</th>
<th>PCSA (cm²)</th>
<th>MV (cm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole muscle</td>
<td>33.4 ± 3.5</td>
<td>2.2 ± 0.3</td>
<td>15.2 ± 6.5</td>
<td>1.41</td>
<td>5.1</td>
</tr>
<tr>
<td>Left</td>
<td>33.4 ± 3.6</td>
<td>2.1 ± 0.2</td>
<td>16.0 ± 6.7</td>
<td>0.78</td>
<td>2.8</td>
</tr>
<tr>
<td>Right</td>
<td>33.3 ± 3.5</td>
<td>2.3 ± 0.3</td>
<td>14.4 ± 6.2</td>
<td>0.63</td>
<td>2.3</td>
</tr>
</tbody>
</table>

The principal fibre bundle orientations, i.e., lines of action, of the left and right geniohyoid muscles were similar (Figure 5.37). In the transverse plane, the fibre bundles were oriented anteromedially; however, the combined line of action of the left and right muscles was directed anteriorly. In the sagittal plane, the combined line of action of the left and right muscles was directed anterosuperiorly.

Figure 5.37. Principal fibre bundle orientations (lines of action) of the left and right geniohyoid muscles. A. Inferior view. B. Lateral view. Green fibre bundles, right geniohyoid; yellow fibre bundles, left geniohyoid; yellow arrows, principal fibre bundle orientation of each muscle; black arrow, combined line of action of right and left geniohyoid muscles.
5.2.3.5 Architectural summary of suprahyoid muscles

Stylohyoid and mylohyoid were found to have the longest mean FBL (46.7 ± 5.5mm and 46.6 ± 9.7mm respectively), and the anterior belly of digastric the shortest (20.5 ± 4.5mm).

Sarcomere length was found to range from 1.8μm to 2.3 μm, except in the anterior and posterior bellies of digastric, which had the shortest SL, ranging from 1.4μm to 1.7μm (Table 5.15).

The mean PA of digastric, stylohyoid and geniohyoid were relatively similar, ranging from 10.7° (anterior belly of digastric) to 16.0° (geniohyoid). Due to its morphological differences, the mean PA of the mylohyoid muscle (63.1 ± 10.9°) was up to six times larger than that of the other suprahyoid muscles.

Table 5.15. Summary of architectural parameters of suprahyoid muscles and their parts.

<table>
<thead>
<tr>
<th>Muscles</th>
<th>FBL (mm)</th>
<th>SL (μm)</th>
<th>PA (°)</th>
<th>PCSA (cm²)</th>
<th>MV (cm³)</th>
<th>Force Index</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Right Digastric</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ant. Digastric</td>
<td>25.5 ± 4.1</td>
<td>1.4 ± 0.1</td>
<td>10.7 ± 5.3</td>
<td>0.95</td>
<td>2.5</td>
<td>0.52</td>
</tr>
<tr>
<td>Post. Digastric</td>
<td>33.1 ± 6.3</td>
<td>1.7 ± 0.1</td>
<td>12.0 ± 5.8</td>
<td>0.86</td>
<td>2.9</td>
<td>0.42</td>
</tr>
<tr>
<td><strong>Left Digastric</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ant. Digastric</td>
<td>25.4 ± 4.1</td>
<td>1.5 ± 0.1</td>
<td>10.7 ± 6.5</td>
<td>0.96</td>
<td>2.5</td>
<td>0.52</td>
</tr>
<tr>
<td>Post. Digastric</td>
<td>26.2 ± 6.2</td>
<td>2.0 ± 0.2</td>
<td>12.9 ± 7.4</td>
<td>1.06</td>
<td>3.0</td>
<td>0.51</td>
</tr>
<tr>
<td><strong>Stylohyoid</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Superficial</td>
<td>43.6 ± 1.6</td>
<td>1.5 ± 0.1</td>
<td>16.2 ± 4.9</td>
<td>0.07</td>
<td>0.3</td>
<td>0.16</td>
</tr>
<tr>
<td>Deep</td>
<td>48.1 ± 6.0</td>
<td>2.3 ± 0.2</td>
<td>8.8 ± 5.1</td>
<td>0.27</td>
<td>1.3</td>
<td>0.23</td>
</tr>
<tr>
<td><strong>Mylohyoid</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anterior</td>
<td>46.6 ± 9.7</td>
<td>2.0 ± 0.2</td>
<td>63.1 ± 10.9</td>
<td>1.40</td>
<td>12.4</td>
<td>0.26</td>
</tr>
<tr>
<td>Middle</td>
<td>44.9 ± 14.0</td>
<td>1.8 ± 0.2</td>
<td>65.1 ± 5.6</td>
<td>0.40</td>
<td>2.1</td>
<td>0.24</td>
</tr>
<tr>
<td>Left</td>
<td>43.7 ± 8.0</td>
<td>2.0 ± 0.2</td>
<td>66.1 ± 6.6</td>
<td>0.46</td>
<td>5.0</td>
<td>0.15</td>
</tr>
<tr>
<td>Right</td>
<td>40.9 ± 8.1</td>
<td>1.9 ± 0.2</td>
<td>72.3 ± 6.6</td>
<td>0.18</td>
<td>2.5</td>
<td>0.10</td>
</tr>
<tr>
<td>Posterior</td>
<td>51.0 ± 4.8</td>
<td>2.1 ± 0.1</td>
<td>56.7 ± 16.6</td>
<td>0.54</td>
<td>5.3</td>
<td>0.18</td>
</tr>
<tr>
<td>Left</td>
<td>46.5 ± 2.5</td>
<td>2.1 ± 0.2</td>
<td>74.4 ± 8.0</td>
<td>0.15</td>
<td>2.3</td>
<td>0.09</td>
</tr>
<tr>
<td>Right</td>
<td>54.3 ± 3.1</td>
<td>2.1 ± 0.1</td>
<td>44.0 ± 6.5</td>
<td>0.39</td>
<td>3.0</td>
<td>0.19</td>
</tr>
<tr>
<td><strong>Geniohyoid</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left</td>
<td>33.4 ± 3.5</td>
<td>2.2 ± 0.3</td>
<td>15.2 ± 6.5</td>
<td>1.41</td>
<td>5.1</td>
<td>0.48</td>
</tr>
<tr>
<td>Right</td>
<td>33.3 ± 3.5</td>
<td>2.3 ± 0.3</td>
<td>14.4 ± 6.2</td>
<td>0.63</td>
<td>2.3</td>
<td>0.36</td>
</tr>
</tbody>
</table>

When the force indices of the suprahyoid muscles were compared, it was found that digastric and geniohyoid had relatively higher indices, with values ranging from 0.42 to 0.52. In
contrast, stylohyoid and mylohyoid had relatively low force indices (0.25 and 0.26, respectively). This suggests that, comparatively, stylohyoid and mylohyoid were designed for excursion, while digastric and geniohyoid were designed for force generation.

Synergism between the suprahyoid muscles as a whole or their component parts could be identified based on their principal fibre bundle orientations and lines of action. For example, the posterior belly of digastric and stylohyoid had similar principal fibre bundle orientations, suggesting that they may be able to work collaboratively to move the hyoid bone posterosuperiorly (Figure 5.38 A and B). Also, the geniohyoid had anterosuperiorly oriented fibre bundles, whereas the anterior belly of digastric had fibre bundles oriented posteroanteriorly, suggesting that in combination, these muscles may produce anterior movement of the hyoid bone with slightly superior displacement (Figure 5.38 C to F).
5.2.4 Infrahyoid muscles

The infrahyoid muscles consist of four muscles that have at least one attachment to the hyoid bone or thyroid cartilage (Figure 5.39).
Figure 5.39. Three-dimensional models of the infrahyoid muscles. A. Sternohyoid, anterior view. B. Omohyoid, anterior view. C. Sternothyroid, anterior view. D. Thyrohyoid, lateral view.

Sternohyoid and omohyoid are superficial and thyrohyoid and sternothyroid lie deep.
- Sternohyoid attached inferiorly to the posterior surface of the manubrium and superiorly to the inferior border of the body of hyoid bone (Figure 5.39 A).

- The superior belly of omohyoid attached to the anterior surface of the body of the hyoid bone and to an intermediate tendon that was anchored by the deep cervical fascia to the clavicle. The inferior belly of omohyoid continued from the intermediate tendon to the superior border of the scapula near the scapular notch (Figure 5.39 B).

- Sternothyroid was found to consist of medial and lateral parts. Inferiorly, the fibre bundles of the medial part originated from the posterior surface of the manubrium, and those of the lateral part from the costal cartilage of the first rib. Superiorly, the medial and lateral parts converged and attached onto the lamina of the thyroid cartilage (Figure 5.39 C).

- Thyrohyoid is a quadrangular muscle attached to the lamina of the thyroid cartilage inferiorly and the lateral surface of the hyoid superiorly. Although similar in attachment, the muscle was divided into superficial and deep parts based on fibre bundle orientation (Figure 5.39 D).

The fibre bundles of all of the infrahyoid muscles were arranged in parallel, spanning almost the entire length of the muscle belly. Due to the parallel fibre bundle arrangement, the PAs of most of the infrahyoid muscles were less than 10°, except for the inferior belly of omohyoid and the superficial part of thyrohyoid, which had slightly larger PAs of about 15° (Table 5.16). Fibre bundle length depended on the attachment sites of the fibre bundles. Sternohyoid had the longest FBL (97.4 ± 1.9mm), which was about three times longer than that
of thyrohyoid. Although thyrohyoid had the shortest mean FBL, its mean SL was longer than that of the other infrahyoid muscles, which had longer fibre bundles.

Table 5.16. Summary of architectural parameters of the infrahyoid muscles and their parts.

<table>
<thead>
<tr>
<th>Muscle</th>
<th>FBL (mm)</th>
<th>SL (μm)</th>
<th>PA (˚)</th>
<th>PCSA (cm²)</th>
<th>MV (cm³)</th>
<th>Force index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sternohyoid</td>
<td>97.4 ± 1.9</td>
<td>1.8 ± 0.3</td>
<td>4.8 ± 3.0</td>
<td>0.5</td>
<td>4.9</td>
<td>0.17</td>
</tr>
<tr>
<td>Omohyoid</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Superior belly</td>
<td>42.3 ± 4.0</td>
<td>1.7 ± 0.1</td>
<td>5.9 ± 1.6</td>
<td>0.21</td>
<td>0.9</td>
<td>0.23</td>
</tr>
<tr>
<td>Inferior belly</td>
<td>69.5 ± 6.1</td>
<td>1.5 ± 0.1</td>
<td>15.5 ± 1.6</td>
<td>0.26</td>
<td>1.9</td>
<td>0.17</td>
</tr>
<tr>
<td>Sternothyroid</td>
<td>72.0 ± 7.8</td>
<td>1.7 ± 0.2</td>
<td>7.9 ± 3.9</td>
<td>0.9</td>
<td>6.6</td>
<td>0.26</td>
</tr>
<tr>
<td>Medial head</td>
<td>70.5 ± 9.8</td>
<td>1.7 ± 0.1</td>
<td>6.7 ± 3.2</td>
<td>0.5</td>
<td>3.4</td>
<td>0.22</td>
</tr>
<tr>
<td>Lateral head</td>
<td>74.3 ± 2.1</td>
<td>1.7 ± 0.2</td>
<td>9.6 ± 4.2</td>
<td>0.4</td>
<td>3.2</td>
<td>0.18</td>
</tr>
<tr>
<td>Thyrohyoid</td>
<td>31.2 ± 6.3</td>
<td>2.0 ± 0.2</td>
<td>9.4 ± 7.3</td>
<td>0.9</td>
<td>2.9</td>
<td>0.44</td>
</tr>
<tr>
<td>Superficial part</td>
<td>35.3 ± 6.5</td>
<td>2.1 ± 0.1</td>
<td>15.2 ± 6.1</td>
<td>0.5</td>
<td>1.6</td>
<td>0.34</td>
</tr>
<tr>
<td>Deep part</td>
<td>30.0 ± 5.7</td>
<td>2.0 ± 0.2</td>
<td>7.6 ± 6.8</td>
<td>0.4</td>
<td>1.3</td>
<td>0.37</td>
</tr>
</tbody>
</table>

Sternothyroid was found to have the largest MV, while thyrohyoid and omohyoid had the smallest. Interestingly, despite having the smallest volume, thyrohyoid had the largest PCSA, the same PCSA as the sternothyroid, which was the largest of the infrahyoid muscles. Therefore, the thyrohyoid muscle had the largest PCSA to MV ratio and the largest force index. The remaining infrahyoid muscles, due to their strap-like morphology, had small force indices.

The principal fibre bundle orientations of the superior and inferior bellies of omohyoid were directed inferolaterally in the coronal plane (Figure 40 A) and inferoposteriorly in the sagittal plane (Figure 5.40 B). The similar alignment of the fibre bundles in both bellies demonstrates the potential for cohesive transmission of forces from the origin of the superior belly to the insertion of the inferior belly.
Figure 5.40. Principal fibre bundle orientations (lines of action) of the superior and inferior bellies of omohyoid. A. Anterior view. B. Lateral view. Yellow fibre bundles, superior belly; red fibre bundles, inferior belly; yellow arrows, principal fibre bundle orientations of the superior and inferior bellies; black arrow, line of action of entire muscle.

In sternothyroid, the principal fibre bundle orientation of the medial part was directed superoinferiorly and that of the lateral part was directed inferolaterally (Figure 5.41). Although both parts may result in inferior movement of the thyroid cartilage, depending on the activation pattern within this muscle, it may enable movement of the thyroid cartilage in multiple axes.
Figure 5.41. Principal fibre bundle orientations (lines of action) of the medial and lateral parts of sternothyroid. A. Anterior view. B. Lateral view. Green fibre bundles, medial part; yellow fibre bundles, lateral part. Yellow arrows, principal fibre bundle orientations of the medial and lateral parts; black arrow, line of action of entire muscle.

Thyrohyoid was found to have two principal fibre bundle orientations, with the superficial part directed inferoanteriorly and the deep part directed inferoposteriorly (Figure 5.42). The two parts of thyrohyoid, similar to those of sternothyroid, may enable fine tuning of movement of the thyroid cartilage.
Figure 5.42. Principal fibre bundle orientations (lines of action) of the superficial and deep parts of thyrohyoid. A. Superficial part. B. Deep part. C. Superficial and deep parts combined. Dark red fibre bundles, superficial part; purple fibre bundles, deep part; yellow arrows, principal fibre bundle orientations of the superficial and deep parts; black arrow, line of action of entire muscle.
Chapter 6

6 Discussion

Using digitization and 3D computer modelling, the musculotendinous architecture of the oro-facial and hyoid muscles was captured and reconstructed three-dimensionally. The 3D model reconstructed from the digitized data was able to capture the geometries of the fibre bundles and the internal and external tendons in situ. From the digitized data, architectural parameters of individual muscles and musculotendinous partitions were quantified. The architectural variations demonstrated in the 3D model and quantified parameters provide a unique opportunity to further investigate the functional capabilities of these muscles and pursue advanced computer modelling leading to more realistic simulation.

The digital atlas described in this study is the only available database that provides 3D coordinate data of the entire oro-facial and hyoid musculotendinous anatomy. The advantages of this technique were described by the candidate in a recent paper (Li et al., 2014): “Since all fibre bundles were digitized from the same specimen, any architectural differences between muscles were real differences and were not caused by interspecimen variation. The digital format allowed us to quantify and visually examine the intricacies of the spatial relationships between muscles, as well as the 3D arrangement of the fibre bundles in relation to their tendons and aponeuroses. The current data demonstrate that even muscles that appear to be morphologically simple, from their superficial appearance, can contain considerable variation in architecture throughout their volumes. The tools and techniques implemented in the current study enabled us to identify and quantify differences between architecturally distinct regions that are often lumped into a single muscle volume.”
Prior to digitization and 3D modelling, most studies were not volumetric and usually sampled a limited number of fibre bundles either from the muscle’s surface or from undocumented locations. Digitization of fibre bundles is done in situ, whereas in earlier cadaveric studies, muscles were removed en block or fibre bundles were removed individually for measurement (Schumacher, 1961; Weijis and Hillen, 1984). The majority of studies reported mean values for architectural parameters, and did not address variation in fibre bundle geometry within a muscle, whereas in this thesis the architectural parameters were quantified at the fibre bundle level. Furthermore, digitization enables capture and subsequent archiving of the 3D data so that the models can be reconstructed and analyzed at any time, even after completion of the dissection. This was not previously an option, since once the dissection had been completed, it was not possible to reassemble the fibre bundles.

Earlier anatomical studies used 2D drawings or photographs and qualitative descriptions to report their findings (Schumacher, 1961; Gaundy et al., 2000; Gassner et al., 2008; Sonoda and Tamatsu, 2008; Nayak et al., 2009). The drawings or photographs only offered a single perspective of the 3D anatomy being studied. In comparison, the 3D model created in this study can be viewed from any angle, and structures can be removed or added to offer unobstructed views of the spatial relationships between the different structures.

Digitization permits the capture of the location, length and trajectory of a large number of fibre bundles in 3D throughout the muscle volume. For example, the number of fibre bundles of the masseter (1265) digitized in this study was 21 times greater than the number of fibre bundles sampled by van Eidjen et al. (1997) (60). Only by digitizing a large number of fibre bundles throughout the volume of each muscle was it possible to capture and quantify the range of architectural variability within and between muscles.
Very few studies have attempted to document the location of aponeuroses/tendons within the muscle volume. This has been difficult since exposure of the deeper aponeuroses/tendons, i.e., not on the surface of the muscle, involves removal of fibre bundles, thus interrupting the relationships of the fibre bundles and the tendinous elements. In contrast, aponeuorses/tendons can be digitized throughout the muscle volume and modelled in conjunction with the digitized fibre bundles, permitting 3D reconstruction of the relationships between the contractile and connective tissue elements.

In vivo examination of muscle geometry can be carried out using ultrasonography. Fibre bundles and aponeuroses/tendons can both be visualized, but this is restricted by wave penetration to superficial musculature and to the measurement plane of the probe. Fibre bundle identification is limited to the measurement plane and therefore can be used to identify isolated fibre bundles throughout the muscle volume, but not to view the entire volume as a whole. Ultrasound studies require detailed knowledge of a muscle’s architecture in order to be able to target key regions in the muscle volume in static or dynamic states.

From the digitized fibre bundle data, architectural parameters can be quantified digitally. For example, when the length of each digitized fibre bundle was computed, the curvature of the fibre bundle could be taken into consideration. In contrast, previously, FBL was measured using a ruler and/or callipers directly from cadaveric specimens (van Eijden et al., 1997; Pearson et al., 2011, 2013). Pennation angle has been measured directly from specimens using a protractor as the angle at the site of attachment of a fibre bundle or to a line connecting the muscle’s attachment sites. Using digitization, it is possible to measure the pennation angle relative to the line of action in 3D space. The line of action can be computed, but not visually defined.
6.1 Modelling

To date, most computer models of the facial, masticatory and/or hyoid musculature have represented individual muscles as a line segment(s) or a 3D volume. For example, Terzopoulos and Waters (1990) and Sifakis et al. (2005) used line segments to represent the muscles of facial expression in a simulation model. In these studies, sphincter muscles (orbicularis oculi and oris) were modelled using multiple line segments linked together to form a polygon that approximates the outer circumference of these muscles. The orientations and attachment sites of the muscles were based on descriptions from the existing literature and anatomical textbooks. In another study, Stavness et al. (2006) developed a line segment model of the masticatory and hyoid muscles based on their attachment sites. The locations and orientations of the muscles were primarily inferred from imaging data. For muscles with broad areas of attachment (i.e., temporalis) or multiple heads (i.e., masseter), 2 to 3 line segments were used to represent the different regions. In contrast, in this thesis, these muscles were reconstructed from in situ digitized data, capturing fibre bundle curvature, interdigitation and architectural variation.

More recently, 3D finite-element (FE) representations of skeletal muscles have been used to attempt simulation of contractile dynamics, such as muscle deformation, surface-to-surface contact and muscular-hydrostasis (Rohler and Pullan, 2007; Cotin, 2008; Stavness et al., 2011; Sanchez et al., 2014). The finite element method is thought to be capable of integrating complex muscle geometry, architecture and tissue properties, but this has not yet been accomplished due to implementation problems. Sanchez et al. (2014) stated, “There has yet to be any work on incorporating detailed fibre fields in skeletal muscles, or tests on the sensitivity of a tissue’s behaviour to variations in the fibre distribution.” The lack of detailed architectural data has also prevented further development of FE muscle models (Rohler and Pullan, 2007; Sanchez et al.,
Rohler and Pullan (2007) stated that “Although it would be desirable to base the FE model fibre geometry on measurements of actual muscle fibres, it was not possible to find applicable data for this.” The database developed as a result of this thesis is the first to include 3D digital data of detailed fibre bundle architecture and tendon geometry of individual muscles. These data are currently being used by our collaborators to begin to build FE models that incorporate complex and realistic fibre bundle and tendon geometry. Thus, the database has filled an important need in model development.

6.2 Muscles of facial expression

No previous study of the architectural parameters of the muscles of facial expression at the fibre bundle level was found. Studies to date have been more general and limited to average length and width of selected muscles (Balogh et al., 1988; Happak et al., 1997). In this thesis, the fibre bundle geometry, including FBL and PA, have been quantified from digitized fibre bundle data, showing variation in the architecture of functionally related muscles. For example, zygomaticus minor had a longer FBL than zygomaticus major, and levator anguli oris was found to have a longer FBL and greater PA than depressor angli oris, with extensive interdigitation of the two muscles.

Interdigitation of the fibre bundles of the muscles of facial expression has been observed in previous studies (Demiryurek et al., 2003; Shim et al., 2008; Yu et al., 2013). For example, interdigitation of zygomaticus major, buccinator, platysma, orbicularis oris, levator anguli oris, mentalis, depressor labii inferioris, depressor anguli oris, and risorius has been reported, but the specific geometry of the interdigitations was not defined (Shim et al., 2008; Al-Hoqail et al., 2009; Yu et al., 2013; Kim et al., 2014). In this thesis, due to 3D digital data collection, the precise location, morphology, interrelationships and interdigitations of the muscles of facial
expression were modelled in 3D as in situ. The fibre bundles of levator/depressor anguli oris were found to interdigitate with the fibre bundles of the upper part of orbicularis oris at the angle of the mouth, whereas the fibre bundles of buccinator interdigitated with the fibre bundles of the lower part of orbicularis oris. Zygomaticus major and minor, levator labii superioris, depressor labii inferioris, LLSAN, and risorius had minimal interdigitation with orbicularis oris.

On morphological comparison of the left and right muscles of facial expression marked asymmetry was noted. For example in the nasal region, greater than 20% differences were observed in the FBL between the right and left nasalis as well as the right and left LLSAN muscles. Asymmetrical findings in muscle architecture and morphology were also observed in the muscles of the oral and orbital regions. Although greater than 20% difference in FBL and variation in the orientation of the line of action were noted between the right and left side, these asymmetries could also be due to use patterns, pathology, injury or deformation of the underlying skeleton. In future studies using digitization and modelling, the detailed architecture of the muscles of facial expression of multiple subjects will be incorporated into the current database to document inter-subject variations. The asymmetrical aspects of facial expression also requires further investigation.

6.3 Muscle architecture

The architectural parameters of the oro-facial and hyoid musculature were quantified for the whole muscle, as well as architecturally distinct regions within the muscle volume. Since the previous literature primarily reported average values of the architectural parameters of each muscle as a whole, this discussion will focus on a comparison of the architectural parameters of individual muscles and muscle groups.
6.3.1 Muscles of mastication

When comparing the architectural parameters computed in this thesis with previous studies, FBL was most consistent across studies and PCSA was most variable. Three previous studies were found that quantified FBL, MV and PCSA (Schumacher, 1961; Weijis and Hillen, 1984; van Eijden et al. 1997), whereas only one study quantified PA (van Eijden et al., 1997).

In all studies, temporalis was found to have the greatest FBL and the medial pterygoid the shortest (Figure 6.1).

![Figure 6.1. Comparison of fibre bundle length of muscles of mastication between the current study and previous literature. Error bars represent the standard deviation across measurements of multiple individuals (previous literature) or within the same individual (current study).](image)

The FBL reported in this thesis for the individual muscles of mastication tended to be longer and demonstrated greater variability than was previously reported. This was likely due to the high density of fibre bundles that were quantified throughout the muscle volume. Furthermore, previous studies included 8 to 30 specimens, but found less variation than this thesis with a
sample size of one specimen. As FBL is indicative a muscle’s excursion capability, the temporalis is expected to have the greatest excursion capability.

Pennation angle was reported in the only previous study (van Eijden et al., 1997) for the parts of each muscle of mastication, rather than for each muscle as a whole. The PA of the parts of the medial pterygoid was found to be consistent between the result by van Eijden et al. (1997) and the current study (Figure 6.2). In contrast, van Eijden et al. (1997) reported a 0° mean PA for the inferior part of the lateral pterygoid, whereas in the current thesis, a mean PA of 12.5° was found. The largest difference in mean PAs was found in temporalis and the deep head of masseter. The mean PA for the deep head of masseter reported in the current study was about 3.6 times greater than that of van Eijden et al. (1997).

![Figure 6.2. Comparison of pennation angle of muscles of mastication between the current study and previous literature. Error bars represent the standard deviation across measurements of multiple individuals (previous literature) or within the same individual (current study).](image)

In this thesis, the PA was calculated at the individual fibre bundle level throughout the muscle volume. In comparison, van Eijden et al. (1997) quantified PA using selected fibre bundles at undisclosed locations in the muscle. This could explain the greater variation in the PA of each muscle observed in the current study.
The volumes of the muscles of mastication were comparable between this thesis and van Eijden et al. (1997). Overall, Schumacher (1961) and Weijis and Hillen (1984) reported much smaller MVs (Figure 6.3). For example, the MV of masseter reported in this thesis was 3.3 times greater than that of Schumacher (1961), and 1.8 times greater than that of Weijis and Hillen (1984). All three of the previous studies weighed the contractile tissue and quantified the volume indirectly by multiplying the mass with a generalized muscle density value (1g/cm$^3$), compared to this thesis, where the MV was computed from the volumetrically digitized fibre bundle fields. The methodology used in previous studies resulted in markedly different muscle volume quantifications between studies, as can be seen in Figure 6.3.

![Figure 6.3. Comparison of muscle volume of muscles of mastication between the current study and previous literature. Error bars represent the standard deviation across measurements of multiple individuals (previous literature) or within the same individual (current study).](image)

Despite the variations in MV found between studies, temporalis was found to have the greatest volume and the medial and lateral pterygoids the smallest.
The masseter and temporalis had a greater PCSA compared to the medial and lateral pterygoids. Across all four studies, the medial pterygoid was found to have a greater PCSA than the lateral pterygoid. In contrast, the masseter muscle in this thesis was found to have the greatest PCSA of all of the muscles of mastication, whereas in all three previous studies, temporalis was reported to have the greatest PCSA. As PCSA is indicative of the force-generating capability, this suggests that the temporalis and masseter muscles have a greater force-generating capability than the pterygoids.

![Figure 6.4. Comparison of physiological cross-sectional area of muscles of mastication between the current study and previous literature. Error bars represent the standard deviation across measurements of multiple individuals (previous literature) or within the same individual (current study).](image)

In all three previous studies, PCSA was calculated by dividing the MV with the mean FBL. In contrast, in this thesis, the PCSA was directly computed from the raw digitized data at the fibre bundle level, taking into account the PA of each fibre bundle.
6.3.2 Suprahyoid muscles

When FBL was compared across all studies of the suprahyoid muscles, stylohyoid was found to have the longest FBL (Figure 6.5), indicating its excursion capability. In this thesis, mylohyoid included all of the fibre bundles in the muscle volume, rather than those of half of the muscle as in previous studies, because the anterior fibre bundles were found to span across the entire floor of the mouth without attaching to a central raphe. It should also be noted that Pearson et al. (2011) measured FBL from photographs.

![Figure 6.5. Comparison of fibre bundle length of suprahyoid muscles between the current study and previous literature. Error bars represent the standard deviation across measurements of multiple individuals (previous literature) or within the same individual (current study).](image)

All of the suprahyoid muscles reported in previous studies had a PA less than 20°. The results of this thesis agree with previous findings, with the exception of mylohyoid (Figure 6.6). The mean PA of mylohyoid reported in this thesis was 63.1°, whereas in van Eijden et al. (1997) and Pearson et al. (2011), the mean PA was 0° and 4.69°, respectively. In this thesis, the line of action of mylohyoid was computationally determined as the resultant vector of average orientations of the fibre bundles, whereas Pearson et al. (2011) used the direction of a line
connecting the centre of the attachment sites on the surface of the muscle. With the methodology developed in this thesis, it was possible to determine the resultant vector according to the definition of the line of action, rather than using external landmarks or centroids within a muscle volume.

Figure 6.6. Comparison of pennation angle of suprathyroid muscles between the current study and previous literature. Error bars represent the standard deviation across measurements of multiple individuals (previous literature) or within the same individual (current study).

The PCSA of all of the suprathyroid muscles, with the exception of mylohyoid, was comparable between this thesis and van Eijden et al. (1997), whereas Pearson et al. (2011) consistently reported a smaller PCSA for all suprathyroid muscles, in particular regarding geniohyoid and the anterior and posterior bellies of digastric (Figure 6.7).
Figure 6.7. Comparison of physiological cross-sectional area between the current study and previous literature. Error bars represent the standard deviation across measurements of multiple individuals (previous literature) or within the same individual (current study).

In the current study and that by Pearson et al. (2011), PA was taken into consideration in the quantification of PCSA, whereas van Eijden et al. (1997) quantified the PCSA of each muscle by dividing the muscle volume by the mean FBL. It is interesting to note that despite only half of the mylohyoid was measured in previous studies, the PCSA reported previously was comparable to this thesis. This may be due to the much greater PA of mylohyoid reported in this thesis. Since the PCSA in this thesis was defined as the sum of the cross-sectional areas of all of the fibre bundles with respect to the muscle’s line of action, and the cosine of 63.1° is 0.45, the PCSA is reduced to 45% of the sum of the cross-sectional areas of all of its fibre bundles.

6.3.3 Infrahyoid muscles

There has been little investigation of the architecture of the infrahyoid muscles in that only one study, Pearson et al. (2013), was found. Pearson et al. (2013) investigated only one infrahyoid muscle, thyrohyoid. The FBL and MV of thyrohyoid found in this thesis were comparable to that reported by Pearson et al. (2013). In contrast, the PA reported in this thesis
was found to be double that of the previous study (Figure 6.9), again, possibly due to measurement of PA from photographs and superficial sampling of fibre bundles.

![Figure 6.8. Comparison of architectural parameters of thyrohyoid muscle between the current study and previous literature. Error bars represent the standard deviation across measurements of multiple individuals (previous literature) or within the same individual (current study).](image)

In addition, this thesis also found greater variability in PA throughout the muscle volume than was previously reported for superficial fibre bundles.

### 6.3.4 Comparison of architecture across muscle groups

The PCSA and FBL of the muscles of mastication, and supra- and infrahyoid muscles are compared in Figure 6.9 to provide an overview of the functional characteristics of each muscle/group, i.e., their excursion and/or force-generating capabilities. The infrahyoid muscles tended to have a greater excursion capability as indicated by their longer FBL, whereas the muscles of mastication had the greatest force-generating capacity as indicated by their large PCSA. The suprahyoid muscles had a comparable PCSA to that of the infrahyoid muscles, but overall, had a shorter FBL.
Figure 6.9. Comparison of the FBL and PCSA of muscles of mastication, and supra- and infrahyoid muscles. L, left side; R, right side.

At the individual muscle level, sternohyoid had the greatest FBL. However, since the omohyoid muscle consisted of two bellies connected in series, the combined FBL of the superior and inferior bellies was found to be 15% longer than sternohyoid. This suggests that omohyoid, upon simultaneous contraction of both its superior and inferior bellies, will produce a greater excursion than sternohyoid. Stylohyoid and mylohyoid, which belong to the suprathyroid muscle group, had a greater FBL than the superior belly of omohyoid and thyrohyoid, which are infrahyoid muscles. The muscles with the greatest force-generating capability, masseter and temporalis, had the greatest PCSA of all of the muscles.
6.4 Limitations

The limitations of this thesis include the small sample size; more specimens would need to be digitized to this level of detail to determine variations between individuals. Further development of the finite element model is necessary, including determination of the accuracy of the placement of the fibre bundle data onto the segmented head and neck skeleton. This did not affect the results of this thesis directly, as the skeletal elements were not used to calculate architectural parameters, but formed a base on which to display the digitized muscles.

6.5 Summary

Based on the results of this thesis, the hypothesis, “The excursion and relative force-generating capabilities, as determined by the muscle architectural parameters, will differ between the oro-facial and hyoid musculature at the muscle group, whole muscle, and muscle partition levels”, was accepted at all levels.

The main objective of this thesis, “To digitize, model and analyse the architecture of the oro-facial and hyoid muscles to be able to compare their force generating and excursion capabilities”, was achieved. At the same time, a comprehensive database of the musculotendinous parameters of these muscles was established for use in modelling, imaging and clinical studies.
Chapter 7

7 Conclusions

1. In this thesis, the first comprehensive 3D model and database of the muscles of facial expression, muscles of mastication, and supra- and infrahyoid muscles was constructed based on tendon/aponeurosis and fibre bundle level digitization. This approach is unique, as it captures the trajectory of thousands of fibre bundles in relation to the complex geometry of internal and external tendons throughout the muscle volume in situ.

2. The 3D model created in this thesis is the only model that is based on digitization and registration of the musculotendinous data to segmented CT scans of the same individual. Forty-eight oro-facial and hyoid muscles are included.

3. A comprehensive database of architectural parameters, including FBL, SL, PA, PCSA and MV, which were quantified in 3D space at the fibre bundle level, was compiled. Sarcomere length could be related to the precise location where the fibre bundle was biopsied, as the site was digitized into the dataset.

4. The functional characteristics, i.e., force-generating and excursion capabilities, of a muscle as a whole and its component parts were determined from the architectural parameters, line(s) of action, and force indices. The functional characteristics were compared between groups of muscles, individual muscles, and musculoaponeurotic partitions. Correlation of the volumetric 3D musculotendinous data obtained in this study with the functional characteristics of a muscle provides a comprehensive approach to assessing the implications of muscle geometry in normal and pathological states.
Chapter 8

8 Future directions

The three-dimensional models and architectural database of the oro-facial and hyoid muscles constructed in this study provide a foundation for the development of more comprehensive and realistic dynamic models to simulate musculoskeletal function with greater fidelity than has previously been possible. Clinically, more specific imaging studies can be defined from these data to study normal and pathological muscle architecture and function in vivo in order to provide insight into the processes following musculoskeletal injury. Four possible future directions are described below.

The 3D data of the oro-facial and hyoid muscles from this thesis are currently being used to develop a state-of-the-art finite element (FE) model in collaboration with our computer science collaborators. The detailed fibre bundle architecture and tendon geometry will be converted into realistic, anatomically based fibre bundle and tendon fields. When embedded into the FE model, it is expected to greatly improve the capability and fidelity of the simulations.

In order to develop a more comprehensive model, the database needs to be expanded to include the soft palate, laryngeal and pharyngeal musculature, and the musculoskeletal structures of the cervical spine. These data would be incorporated into the finite element model for dynamic simulation of mastication, swallowing and speaking. Validation of the model’s ability to realistically simulate dynamic function is important and may be carried out using in vivo imaging such as ultrasonography and fluoroscopy, and functional studies including electromyography and motion capture.
To add a motor control component to the dynamic model, a comprehensive 3D map of the extra- and intramuscular innervation of each muscle is necessary. This could be accomplished by digitizing the nerve distribution throughout the muscle volume, which would enable correlation of musculoaponeurotic partitioning with innervation. The 3D innervation map could then be incorporated into the FE model to provide neurally based contraction at the level of the whole muscle, muscle group, or neuromuscular partition.

Clinically, it is not understood how architectural parameters are affected at the fibre bundle level in individuals with muscular pathology, e.g., tendon tears. Detailed ultrasound protocols could be developed based on the results of this thesis to investigate changes in musculotendinous architecture in pathological states. In addition to identifying alterations in musculotendinous architecture, the results could be used to create patient-specific FE models that could be used to simulate the condition and provide a mechanism to test surgical and rehabilitation interventions.
References


Copyright Acknowledgements

Thesis / Dissertation Reuse

LWW grants permission for a maximum of 3 figures or tables without charge, provided that the material is for limited thesis/dissertation distribution. If you are required to post your thesis/dissertation on the university website/intranet/library reserve, access to it must be password-protected. Should you wish to publish your paper in the future, you will need to reapply for permission to use our material.
Permission for use of figures

Anne Agur <anne.agur@gmail.com>  Tue, Jun 10, 2014 at 10:16 AM
To: David Cormack <dhcormack@rogers.com>

Dear Dr. David Cormack,
Kate Sauks, my PhD student, is requesting use of these figures (from your out of print books and Dr. Ham's as you continued as the author of his book) for use in her PhD thesis on the effect of PRP and Traumeel on tendon healing.

1) Figures from Ham's Histology 9th Ed. Author: D. Cormack.
   - Fig 15-4, pg. 391
   - Fig 11-1, pg. 266

2) Figures from Essential Histology, 2nd Ed. Author: D. Cormack
   - Fig 10.1, pg. 239
   - Fig 10.2, pg. 240
   - Fig 10.4, pg. 241
   - Fig 8.1, pg. 176 --> I still need the original file for this figure

3) Figure from Histology, 7th Ed. Author: A. Ham
   - Fig 18-13, pg. 540

Thank you for your consideration.
Anne Agur
--

Anne Agur, BSc(OT), MSc, PhD
Professor, Division of Anatomy, Department of Surgery
1 King's College Circle
Medical Sciences Building Rm 1158
Toronto, Ontario, Canada
M5S 1A8
416-978-8855
anne.agur@utoronto.ca

David Cormack <dhcormack@rogers.com>  Tue, Jun 10, 2014 at 12:25 PM
To: Anne Agur <anne.agur@gmail.com>

Dear Dr. Agur,

The use of these figures in all theses by your graduate students (including Kate Sauks) is hereby approved, as from June 10, 2014. If further illustrations are required, just let me know. Sorry I do not have the original file for Fig. 8-1, but LLW may have it. Otherwise, I would use a scan -- it would look just as good.
Very glad to be of assistance in this matter,

Sincerely,

David H. Cormack, Ph.D.

Professor Emeritus
Division of Anatomy,
Department of Surgery,
University of Toronto

Sent from my iPad

Anne Agur <anne.agur@gmail.com> Tue, Jun 10, 2014 at 1:22 PM
To: Kate Sauks <kate.sauks@utoronto.ca>

[Quoted text hidden]
Recently you requested personal assistance from our on-line support center. Below is a summary of your request and our response.

If you are receiving this in response to a request you made, a summary is below. If you have not made a request, the following is a communication on behalf of your LWW Sales Representative.

Thank you for allowing us to be of service to you.

To access your question from our support site, click here.

Subject
Michael H. Ross; Wojciech Pawlina Book Permissions Request

Discussion Thread
Response Via Email (Caren Erlichman) 01/22/2015 04:42 PM

Hello Mr. Li.

Thank you for contacting us. We do allow material from Ross to be borrowed, but for a thesis we can allow you 3 figures at no cost. Can you please tell me which 3 you would like to use and I can put together a letter for you?

Thank you.

Sincerely,

Caren Erlichman

Customer By Email 01/22/2015 03:57 PM

<table>
<thead>
<tr>
<th>Question</th>
<th>Answer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Name</td>
<td>Mr. Zhi Li</td>
</tr>
<tr>
<td>Institution Name</td>
<td>University of Toronto</td>
</tr>
<tr>
<td>Street Address</td>
<td>1 King’s College Circle</td>
</tr>
</tbody>
</table>

https://jot51030.outlook.com/owa/jotprojection.aspx