

Faculdade de Engenharia da Universidade do Porto



**Computational Structural Simulation of Chitosan
Tubes Used for the Regenerating Peripheral
Nerve Tissues**

Tomé Mendes Albuquerque

Dissertação realizada no âmbito do
Mestrado em Engenharia Biomédica

Orientador: Prof. Jorge Américo Oliveira Pinto Belinha
Co-orientador: Prof. Renato Manuel Natal Jorge

June, 2019

© Tomé Mendes Albuquerque, 2019

Resumo

As lesões em nervos periféricos têm um impacto enorme não apenas nos indivíduos, mas também na sociedade em geral. Estas incorporam a principal causa de incapacidade e morbidade em todo o mundo e acarretam custos extremamente elevados anualmente.

Atualmente existem duas abordagens distintas para a reparação de uma lesão no sistema nervoso periférico: sutura de ponta a ponta e “bridging”. A primeira estratégia é a mais limitante, pois só pode ser usada quando o paciente tem uma pequena falha no tecido nervoso. A segunda consiste no preenchimento de fendas nervosas de maior comprimento crítico com um enxerto de nervo autólogo. Embora este método seja o mais reconhecido atualmente, este está longe de ser ideal e muitas vezes é insatisfatório devido a vários fatores, como a morbidade neurológica no local doador e também a possibilidade de formação de neuroma. Para superar as complicações do uso dos enxertos de nervo autólogo, a Intercalação do nervo com um tubo acelular artificial ou aloenxerto celular, é uma alternativa viável. Surgem assim os tubos guias de quitosano, devido às suas excelentes propriedades biológicas.

Assim sendo, é essencial estudar as propriedades biomecânicas dos tubos de quitosano para melhorar o procedimento de reparação e regeneração dos nervos periféricos, de modo a garantir a resistência mecânica ideal do tubo de quitosano. O objetivo referido anteriormente pode ser alcançado usando diferentes desenhos do tubo, variando a sua geometria interna e externa.

Na presente dissertação, foi efetuado o estudo da resposta estrutural biomecânica de tubos de quitosano usados na reparação nervosa periférica. Para tal, foram usadas técnicas computacionais de discretização avançadas como os métodos sem malha (RPIM e NNRPIM) e FEM. Através destes métodos foi possível obter conclusões práticas significativas acerca das dimensões e espessuras ideais do tubo através de análises elasto-estáticas. Adicionalmente, os resultados adquiridos para os métodos sem malha aproximam-se dos resultados obtidos para o FEM.

Abstract

Lesions on peripheral nerves have a considerable impact not only on individuals but also on society. These are the main cause of disability and morbidity around the world and entail extremely high costs annually.

There are currently two distinct approaches to repair a peripheral nervous system injury: end-to-end suturing and bridging. The first strategy is the most limiting since it can only be used when the patient has a short gap in the nervous tissue. The second approach consists of filling nerve gaps of greater critical length with an autologous nerve graft. Although this method is the most recognized nowadays, it is far from ideal and is often unsatisfactory due to several factors, such as neurological morbidity in the donor site and the possibility of neuroma formation. To overcome the complications of using autologous nerve grafts, nerve interposition with an artificial acellular tube or cell allograft is a viable alternative. The chitosan guide tubes thus arise due to their excellent biological properties.

So, it is essential to study the biomechanical properties of chitosan tubes to improve the repair and regeneration procedure of the peripheral nerves, to guarantee the ideal mechanical resistance of the chitosan tube. To achieve the previously mentioned objective different designs of the chitosan tube can be used.

In the present dissertation, the biomechanical structural response of chitosan tubes used in peripheral nerve repair was studied. For this, advanced discretizing computational techniques such as non-mesh methods (RPIM and NNRPIM) and FEM were used. Through these methods, it was possible to obtain significant practical conclusions about the ideal dimensions and thickness of the tube through elasto-static analysis. In addition, the results obtained for the non-knit methods approximate the results obtained for the FEM.

Acknowledgements

The realisation of this work was only possible with the support and collaboration of several people. To all who directly or indirectly participated in this work, I would like to express my gratitude.

A big thank you to Prof. Jorge Belinha not only for the opportunity he gave to me to carry out this project but also for all his support, help and friendship. Thank you for the weekly meetings, the councils and your dedication.

To my friends, for working together, for sharing knowledge, for support. They have always been with me and it was with you that I shared some of the best experiences that I will remember forever.

A huge thanks to my parents, Goretti and Manuel, for all the support, affection, motivation, strength and patience, even in my most frustrating moments. This step completed successfully is thanks to you who have always encouraged me to study, to go further and to give the best of me.

My special thanks to my grandparents for all the affection and comfort they give me every day.

A special thank you to Marta. To you, I thank for all your support, affection and for always being so understanding. For never giving up on me and for all the 'Tomé vai estudar'. I love you

Tomé Mendes Albuquerque

Institutional Acknowledgements

The author truly acknowledges the work conditions provided by the Applied Mechanics Division (SMAp) of the department of mechanical engineering (DEMec) of Faculty of Engineering of the University of Porto (FEUP), and by the MIT-Portugal project “MIT-EXPL/ISF/0084/2017”, funded by Massachusetts Institute of Technology (USA) and “Ministério da Ciência, Tecnologia e Ensino Superior - Fundação para a Ciência e a Tecnologia” (Portugal).

Additionally, the authors gratefully acknowledge the funding of Project NORTE-01-0145-FEDER-000022 - SciTech - Science and Technology for Competitive and Sustainable Industries, cofinanced by Programa Operacional Regional do Norte (NORTE2020), through Fundo Europeu de Desenvolvimento Regional (FEDER).

Finally, the author acknowledges the synergetic collaboration with the collaborators of “Computational Mechanics Research Laboratory CMech-Lab” (ISEP/FEUP/INEGI), and its director, Prof.Dr. Jorge Belinha, and its senior advisors, Prof.Dr. Renato Natal Jorge and Prof.Dr. Lúcia Dinis.

Contents

Resumo	iii
Abstract.....	v
Acknowledgements	vii
Institutional Acknowledgements	viii
Contents	x
List of figures.....	xii
List of tables.....	xvi
Abbreviations and Symbols	xviii
Chapter 1.....	21
Introduction.....	21
1.1 - Motivation.....	22
1.2 - Objectives.....	23
1.3 - Document Structure.....	23
Chapter 2.....	25
Peripheral Nerve.....	25
2.1 - Peripheral Nervous System.....	26
2.1.1 - Spinal Nerves - Structure and Nerve plexus	28
2.1.2 - Nerve fibers and nerve sheaths.....	36
2.1.3 - Peripheral nerve, Connective tissue structure and vessel supply on peripheral nervous system.....	42
2.2 - Types of injuries and accidents	46
2.3 - Peripheral nerve regeneration and therapy	48
2.4 - Mechanical properties of peripheral nerves	49
2.5 - Constitutive Models	51
Chapter 3.....	54
Chitosan Biomaterial	54
3.1 - Chitosan properties and manufacturing process	55
3.2 - Mechanical properties of Chitosan	58

3.3 -Constitutive models	59
Chapter 4.....	62
Numerical Methods	62
4.1 - FEM	62
4.2 - Meshless Methods	64
4.2.1 - Common procedure in Meshless	65
4.2.2 - Nodal Connectivity	66
4.2.2.1 - RPIM	66
4.2.2.2 - NNRPIM.....	67
4.2.3 - Numerical Integration	70
4.2.3.1 - RPIM	70
4.2.3.1 - NNRPIM.....	74
4.2.4 - Interpolation Functions.....	76
4.3 - Solid Mechanics	79
4.3.1 - Basic Concepts	79
4.3.2 - Strong form and Weak form	81
4.3.2.1 - Galerkin Weak form	81
4.3.3 - Discrete equation system	84
Chapter 5.....	87
Numerical methods applied to nerve tissue.....	87
5.1 - FEM	87
5.1 .1 - Geometrical Models	88
5.1 .1.1 - Two-dimensional	88
5.1 .1.2 - Three-dimensional	89
5.1.2 - Boundary conditions	90
5.1.3 - Constitutive models.....	90
5.1.4 - Structural analysis.....	90
5.2 - Meshless	90
Chapter 6.....	93
Preliminary Research Work	93
6.1 - Construction of the model	93
6.2 - Model Proprieties and Analysis	94
Chapter 7.....	102
Elasto-static numerical analysis.....	102
7.1- Study of convergence	103
7.2 - Study of chitosan tube geometry	111
7.3 - Study of chitosan tube external geometry	118
Chapter 8.....	124
Conclusions and Future Work	124
References	128

List of figures

Figure 1 - The health care costs (blue); costs of lost production (red) - place of injury (a) by labour activity category (b) [2].	26
Figure 2 - Simplified representation of peripheral nervous system.	27
Figure 3 - Location of cranial nerves and the efferent and afferent fibers [25].	28
Figure 4 - Representative scheme of: (a) the source of the roots from the cord, their junction and the appearance of the nerve from the spinal cord. (b) The partition of the spinal nerve (cervical region) into anterior and posterior primary rami [23].	29
Figure 5 - (a) The right brachial plexus; (b) The anterior perspective of the right elbow [23].	30
Figure 6 - The right : (a) musculocutaneous nerve; (b) radial nerve; (c) median nerve; (d) ulnar nerve [23].	31
Figure 7 - (a) Median and ulnar nerves in the hand; (b) Nerves on the anterior aspect of the right lower limb; (c) nerves on the posterior perspective of the right limb [23].	32
Figure 8 - Left - Right sciatic nerve and its main components in the lower limb; Right - Femoral and sacral plexuses and the ganglionated sympathetic chain [23].	33
Figure 9 - Organization of the different nerve plexuses [24].	34
Figure 10 - Dermatomes of anterior and posterior surfaces in human body [24].	35
Figure 11 - Neuron Structure [24].	37
Figure 12 - Representation of the three different types of neurons: a) multipolar neuron; b) bipolar neuron; c) pseudounipolar neuron.	38
Figure 13 - 3D structure of a nerve fibre [24].	39
Figure 14 - Different types of nerve fibres.	41
Figure 15 - Schematic of the composition of a peripheral nerve [24].	42
Figure 16 - Simplified representation of the different connective tissues on the peripheral nerve [24].	44
Figure 17 - Spatial distribution and disposition of blood vessels in nerves (vasa nervorum). ...	45

Figure 18 - Intraneural microvasculature structure [24].....	45
Figure 19 - Schematic examples of nerve fibre lesions and conductivity speed graphs [24]. ...	47
Figure 20 - Regenerative process sequence using an NGC hollow [11].	49
Figure 21 - Diverse types of stress applied on a peripheral nerve [43].....	50
Figure 22 - Chemical structure of Chitosan [50].....	55
Figure 23 - Adhesion of PC12 cells to the different types of materials: (a) in serum free medium, (b) in medium containing 5% serum [60].	57
Figure 24 - Tensile stress-strain curve for a porous chitosan film [61].....	58
Figure 25 - Conception of a mesh using FEM.	63
Figure 26 - Influence of the mesh on the discretization error [73].	63
Figure 27 - Nodal discretization example: a) Solid domain. b) regular nodal discretization c) Irregular nodal discretization [20].	65
Figure 28 - Diverse types of influence domains: (a) Fixed rectangular shaped (b) Fixed circular shaped c Flexible circular shaped [20].....	67
Figure 29 -(a) Initial nodal set of possible neighbour nodes (b) First trial plane (c) Second ...	68
Figure 30 - (a) First-degree influence-cell; (b) Second-degree influence-cell [20].	70
Figure 31 - (a) Fitted background mesh; (b) Regular grid integration mesh [20].	70
Figure 32 - (a) Early grid-cell; (b) Isoparametric square with integration points; (c) Initial cell with integration points [20].	71
Figure 33 - Early Voronoï diagram. b Delaunay triangulation [20]..	74
Figure 34 - (a) Voronoï cell and the corresponding intersection points, P_{li} ; (b) Middle points, M_{li} , and the respective generated quadrilaterals; (c) Quadrilateral [20]..	74
Figure 35 - Voronoï cell and the corresponding intersection points, P_{li} ; (b) Middle points, M_{li} , and the respective generated triangles; (c) Triangle [20].	75
Figure 36 - Triangular and quadrilateral shapes and the corresponding integration points x_i [20]..	75
Figure 37 - Triangular and quadrilateral shapes - division, process in sub-cells and respective integration points x_i by the Gauss-Legendre integration scheme [20].	76
Figure 38 - Continuous solid body subject to volume and external forces [20].	82
Figure 39 - Amount of papers published through the years founded in a research on Scopus database (www.scopus.com) assuming the keywords mentioned before.	87
Figure 40 - Distribution of normal stress (σ_{yy}) in the deformed nerve ultrastructure [88]. ...	88
Figure 41 - Example of a 3D FE model [92].	89

Figure 42 - Evolution of the model: (a) 3d model built in SolidWorks®; (b) After smooth the model in Meshmixer®; (c) Final model in FEMAS after the creation of the mesh in FEMAP®.	94
Figure 43 - Peripheral nerve bifurcation bidimensional model with main dimensions.	94
Figure 44 - Peripheral nerve bifurcation 3D model.	95
Figure 45 - Boundary conditions on: (a) 2D model and (b) 3D model.	96
Figure 46 - Force applied in the 2D and 3D model.	96
Figure 47 - Selected points to displacement analysis in the 2D (a) and 3D (b) model.	97
Figure 48 - (a) Dimensions of the model 1 in millimetres (mm); (b) model 1 patch.....	103
Figure 49 - Boundary conditions and forces applied in the 3D model 1.	104
Figure 50 - Total displacement as a function of the nodal discretization of the 3D geometric model.	104
Figure 51 - Stress (σ_{xx}) on point A and B as a function of the nodal discretization of the 3D geometric model 1.	105
Figure 52 - (a) Dimensions of the chitosan tube in millimetres (mm); (b) chitosan tube model 2 with the three different patches.	106
Figure 53 - Model 2 with smaller (left) and larger (right) number of nodes, used in the convergence study.	106
Figure 54 - Boundary conditions imposed on model 2.....	107
Figure 55 - Forces applied in the 3D models.	108
Figure 56 - Total displacement as a function of the nodal discretization of the 3D geometric model.	109
Figure 57 - Effective von Mises stress on point A and B as a function of the nodal discretization of the 3D geometric model.	109
Figure 58 - schematic of the construction of the different chitosan tube models.....	112
Figure 59 - (a),(b),(c) - FEM results for displacement (v) vs nerve gap and nerve suture lengths. (d),(e),(f) - RPIM results for displacement (v) vs nerve gap and nerve suture lengths.	113
Figure 60 - (a),(b),(c) - FEM results for effective von Mises stress vs nerve gap and nerve suture lengths. (d),(e),(f) - RPIM results for effective von Mises stress vs nerve gap and nerve suture lengths.	114
Figure 61 - FEM results for displacement (v) ((a) and (c)) and effective von Mises stress ((b) and (d)) for the different thickness vs nerve gap or nerve suture lengths.	115
Figure 62 - RPIM results for displacement (v) ((a) and (c)) and effective von Mises stress ((b) and (d)) for the different thickness vs nerve gap or nerve suture lengths.	116
Figure 63 - Approximation functions (more transparent) for displacement (v) for the three different thickness of the chitosan tube wall.	117

Figure 64 - (a) schematic of the construction of the chitosan tube models. (b) The appearance of the total chitosan tube.	118
Figure 65 -FEM results for effective von Mises stress in function of the different thickness in the top of the chitosan tube.	119
Figure 66 -RPIM results for effective von Mises stress in function of the different thickness in the top of the chitosan tube.	120
Figure 67 -FEM results for total displacement in function of the different thickness in the top of the chitosan tube.	120
Figure 68 - RPIM results for the total displacement in function of the different thickness in the top of the chitosan tube.	121

List of tables

Table 1 - Gasser and Erlanger classification according to structure and physiology of nerve fibers [24].	41
Table 2 - Schematic correspondence between Sunderland's and Seddon's classification systems [24].	46
Table 3 - Summary of tensile properties of chitosan films [66].	59
Table 4 - Integration points and corresponding weights for triangles isoparametric cells.	72
Table 5 - Integration points and matching weights for quadrilaterals isoparametric cells.	73
Table 6 - Mechanical properties of the material.	95
Table 7 - Total displacement (mm) for the selected points (PA and PB) in the two models ($ u $).	97
Table 8 - Von Mises effective von Mises stress of both models.	98
Table 9 - σ_{11} stress of both models.	98
Table 10 - σ_{22} stress of both models.	99
Table 11 - σ_{33} stress of 3D model.	99
Table 12 - Different nodal density used in model 1 on the convergence study.	103
Table 13 - Mechanical proprieties of the material.	104
Table 14 - Different nodal density in the models used in convergence study.	107
Table 15 - Mechanical proprieties of the materials.	107
Table 16 - Results obtained in FEM and RPIM structural analysis in the second convergence study for effective von Mises stress, σ_{11} and σ_{33}	110
Table 17 - Results obtained in NNRPIM structural analysis in the second convergence study for effective von Mises stress, σ_{11} and σ_{33}	111

Table 18 - Values of the parameters used in the approximation functions of displacement (v).	118
Table 19 - Isomaps of the total displacement and effective von Mises stress of the models submitted to FEM and RPIM analysis.	122

Abbreviations and Symbols

Abbreviations

2D	Two-dimensional
3D	Three-dimensional
CAD	Computer Aided Design
CNS	Central Nervous System
CT	Computed Tomography
DA	Degree of Acetylation
DD	Degree of Deacetylation
DEM	Diffuse Element Method
DICOM	Digital Imaging and Communications in Medicine
EFGM	Element Free Galerkin Method
FEM	Finite Element Method
FEMAP	Finite Element and Postprocessing
FEMAS	Finite Element and Meshless Analysis Software
GAG	Glycosaminoglycans
MLS	Moving Least Square
MR	magnetic resonance
NEM	Natural Element Method
NGC	Nerve Guidance Conduit
NNRPIM	Natural Neighbour Radial Point Interpolation Method
NREM	Natural Radial Element Method
OCT	Optical Coherence Tomography
PNS	Peripheral Nervous system
RBF	Radial Basis Function
RKPM	Reproducing Kernel Particle Method
RPI	Radial Point Interpolators
RPIM	Radial Point Interpolation Method

SCs Schwann cells

Symbols

E Young's modulus

ν Poisson ration

Chapter 1

Introduction

Peripheral nerve injuries have a huge impact not only on the individual subjects but also on the society. They embody the major cause of disability and morbidity worldwide and entail extremely high costs annually [1]-[3]. The injuries are one of the complications of trauma, affecting the extremities of 3-10% patients, that hang on the mechanism of trauma, but some patients acquire life-long disability [1], [4], [5]. The most common trauma is in the upper extremity that patients account for 10-30% of the attendance to an emergency department in the hospital. Male gender is more associated with peripheral nerve injuries due to vehicle accidents [2], [6]. Hand and arm injuries had a direct impact not only on the daily patient life but also on work capability and leisure activities, leading to substantial costs to the society, for example, health care costs and lost production costs [2], [7].

The major challenge in reconstructive surgery and regenerative medicine nowadays is the treatment of peripheral nerve injuries: transection and laceration. Even though the peripheral nervous system (PNS) has the capacity to regenerate, in the presence of an appropriate environment, the degree of functional recovery depends of several factors, such as the health condition and the age of the patient, the location and length of the injury, the type of nerve, the time between the accident and the surgical intervention [8]-[10]. After the injury, our body starts immediately the regenerative process [6].

Nowadays, there are two different approaches to repair an injury on the PNS: bridging and end-to-end suturing. In the first one, bridging nerve gaps of a critical length ($>3\text{cm}$) with an autologous nerve graft (autograft) remains the gold standard for repairing non-suturable injuries on PNS [11]. However, this method is far from ideal and is often unsatisfactory due to several factors: the autologous nerve donor may be in the origin of neurological morbidity at the donor site, including probably a neuroma formation; another disadvantage is the need to sacrifice a health nerve in a second chirurgical intervention to crop the nerve graft; grafting motor nerves with sensory nerves is another disadvantage due to the anatomic nerve differences [12]-[15]. The second strategy, end-to-end suturing, can only be used when the patient has a small nerve gap ($<5\text{ mm}$) and the tension-free coaptation among the proximal and distal nerve end is attainable.

To overcome the autologous nerve grafts complications the Interposition of nerve scaffold whether artificial acellular conduit or a cellular allograft is a viable alternative [15].

To achieve the disadvantages of autografts and replace them as a part of a peripheral nerve regeneration approach, the scaffold should meet the following five requirements: (1) biocompatibility with the tissues, (2) nonimmunogenicity and nontoxicity (3) biodegradability at optimal rate, (4) suitable porosity and morphology and (5) optimal mechanical properties [16]. The scaffold in most of the cases is made of natural (e.g., collagen) or synthetic polymers (e.g., silicone).

In this situation, chitosan is an attractive candidate in a broad spectrum of applications. This biomaterial is cheap because the source material for chitosan is chitin, an important component of the exoskeleton in animals, and it is one of the most abundant organic materials on the planet. Chitosan has exclusive biological properties counting biocompatibility, biodegradability, nontoxicity, antitumoral, anticholesteremic, extraordinary affinity to proteins, antibacterial, hemostatic and fungistatic properties [3], [16]-[18]. The use of nerve guidance channels (NGC) made with chitosan, sutured among the proximal and distal nerve ends, has been actively pursued to remove the need for the second surgical procedure and to acquire better regenerative outcomes in comparison to the autologous nerve graft. This method causes less surgical trauma at the repair site of the patient and decreases the probability of a neuroma formation [15][19].

In this context, it is important to study the biomechanical behavior of NGC's made with chitosan, so numerical-experimental approaches serve as comprehensive methodologies of in vitro research for the study of this complex behavior, 3D segmentation techniques allow to construct the numerical model. This project purposes to use advanced discretization computational techniques, such as meshless methods to study the biomechanical behavior of NGC's, there is a gap in the literature on this subject.

The 3D model of the chitosan tube can be built in CAD software. In this way, the 3D complete model can be studied using a meshless academic software, in which some important parameters can be obtained and analyzed such as variable fields (displacement/stress/strain fields).

One of the goals in this work consists in to demonstrate that meshless methods can bring precise solutions for the most complex problems, being an efficient alternative, according to the literature [20].

1.1 - Motivation

The use of NGC's to overcome the autologous nerve grafts complications is a viable alternative. Thus, it will be extremely important to study these artificial guides mechanically so that their characteristics can be improved.

Through computational biomechanics it is possible to do a numerical simulation of complex problems and, in this circumstance, may help to study the biomechanical behavior of NGC's when subject to mechanical stress. Although the Finite Element Method (FEM) is one of the most used numeric methods in the world, other different methods have been successfully used as a viable alternative, such as meshless methods.

Another important motivation factor is to improve the state-of-the-art of meshless methods because there is a gap in the literature about this subject and recognize the level of

performance of these methods. The number of papers about meshless is low and in the case of this study are inexistent.

1.2 - Objectives

One objective of this work is to use numerical methods to analyze structurally chitosan tubes submitted to benchmark structural tests, such as the flexion test. The numerical methods under focus are the finite element method (FEM), the radial point interpolation method (RPIM) and with natural neighbor radial point interpolation method (NNRPIM).

Another important goal is the corroboration of the meshless methods as a viable way of simulating and studying the behavior of NGC's.

There are others secondary objectives such as the comparison between the results obtained experimentally and the computational solutions, to authenticate the mathematical model used; different test conditions of the chitosan tubes to achieve which as better mechanical proprieties;

Nevertheless, in order to comprehend all the topics involving the dissertation, first an extensive survey is performed in the following topics:

- Peripheral nerve (physiology)
- Chitosan biomaterial (compositions and mechanical properties).
- Discrete numerical techniques (mathematical formulation and state-of-the-art)

Furthermore, the work developed along the development of the present dissertation intended also to achieve a proficient level, as user, of the following software:

- 3D-Slicer - a freeware capable to produce STL files from CT medical images;
- FEMAP (Siemens PLM Software, student version) - a commercial software creates virtual geometrical models and their respective meshes;
- FEMAS® (Finite Element and Meshless Analysis Software - cmech.webs.com) - a freeware academic program which allows the structural analysis of computational models.

1.3 - Document Structure

This dissertation is organized in eight different chapters. Chapter 1 presents a brief introduction, the main objectives and the motivation that led to the development of this work. In Chapter 2, it is presented not only anatomic concepts of the peripheral nervous system but also microstructural aspects, types of accidents and injuries that can occur and the biomechanical properties of the peripheral nerve. Chapter 3 is about chitosan biomaterial, it addresses several themes, such as the method of manufacturing chitosan, mechanical properties and some applications of the chitosan.

Chapter 4 is divided into three different parts, initially, are presented with a brief description of the finite element method (FEM) and some its advantages and disadvantages. The second part of this chapter is based on a short explanation of two distinct meshless methods: RPIM and NRRPIM. The last part of this chapter is focused on solids mechanics where stress, strain, and displacement relationships are studied. The Chapter 5 consists in the state-of-the-art of chitosan and peripheral nerves numerical analyses founded in literature.

The next chapter, Chapter 6, consists in the preliminary work that was done and all the obtained results. In Chapter 7 it is presented the Elasto-static numerical analysis which contain the study of convergence and study of chitosan tube geometry internal and external. The last chapter, Chapter 8, contains the conclusions in which are exposed the main conclusions withdrawn from the results and future works.

Chapter 2

Peripheral Nerve

Peripheral nerve injuries have a massive impact on the daily life of millions of people, embodying the major cause of disability and morbidity across the entire world. [1]-[3]. The percentage of patients with trauma in the upper extremity account for 10-30% of the attendance to an accident and emergency department; it is the most common trauma affecting in most of the cases young men. Hand and arm injuries affect the daily patient routine in several aspects: work competence, leisure activities, and other daily activities. Peripheral nerve injuries are associated with high monetary costs annually due to several factors such as the loss of production due to work capacity affected of the patient and health care costs [2]. Outside of work is considered the favorite place for nerve injury due to several causes: machines, glass, saw and other different sources. The total costs in patients injured at work are 34% higher when compared with patients injured in other activities. This higher percentage is associated with costs of lost production due that the patients that worked full time before the injury is now impaired. However, the health care costs in patients at part-time work before the injury were +66% higher [2]. Another important factor is the influence of patients age on health care costs; the cost increases with age. Figure 1 shows in euros the average of health care costs (blue) and costs of lost production (red), divided by the place of injury (a) or by labor activity group (b) [2].

The thousands of euros that are spent in peripheral nerve injuries by the patients on a global scale, these numbers reach millions and millions spent every year. Thus, it is very important to study and find solutions and improve existing ones to treat peripheral nerve injuries. Most of the injuries in the human peripheral nervous system can be divided into three different categories: amputations (29%), complex injuries (i.e., a combination of fractures, tendon, vessel and nerve injuries; 40%) and major nerve injuries [2].

In this chapter are presented not only anatomic concepts of the peripheral nervous system but also microstructural aspects, types of accidents and injuries that can occur and the biomechanical properties of the peripheral nerve.

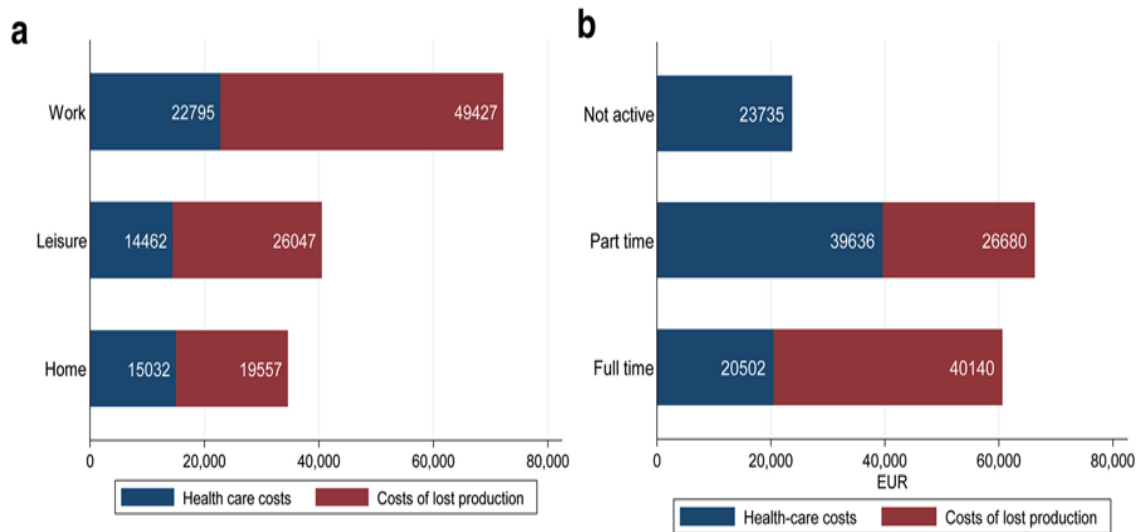


Figure 1 - The health care costs (blue); costs of lost production (red) - place of injury (a) by labour activity category (b) [2].

2.1 - Peripheral Nervous System

The nervous system can be divided into two different parts: the peripheral nervous system (PNS) and central nervous system (CNS). They differ in their function and physiology; the PNS contains afferent sensory nerves that receive information from the external environment and carry signals to the brain and spinal cord, which constitute the central nervous system (CNS), then efferent motor nerves conveying instructions from it. The CNS has an integrative function, and it is connected up by a giant number of interneurons [21], [22].

The PNS has the function of connecting all organs and tissues with the CNS; this system can be divided into two different groups according to its anatomic, functional and structural properties to its organ targets:

(1) Cerebrospinal nervous system: Composed of 12 pairs of cranial nerves (encephalon) and 31 pairs of spinal nerves (spinal cord), this system has the function to connect the brain and spinal cord to the external world and all the sensory impulses that can occur. The cranial nerves are located from the base of the skull to the head, neck, and trunk, on the other hand, the spinal nerves pass through the intervertebral foramen, innervating the trunk, legs, and arms.

(2) Autonomic nervous system: This system is continuously in functioning independently of the conscious effort, can be separated into three different divisions: the sympathetic, the parasympathetic and the enteric. In each, pre-ganglionic fibers arise from cells in the brain stem or spinal cord. These relay in ganglia to post-ganglionic fibers innervating cardiac and smooth muscle, and glands. Sympathetic and parasympathetic divisions supply most of the viscera. The cell bodies of the enteric system are limited to the wall of the bowel [23], [24].

Figure 2 represents a simplified representation of the peripheral nervous system divided by the major groups that were mentioned before.

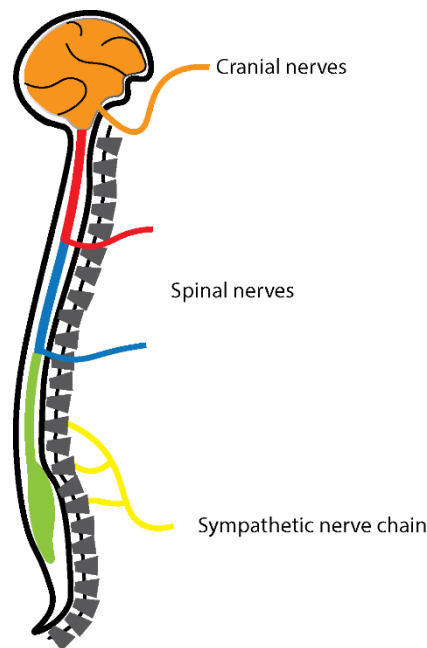


Figure 2 - Simplified representation of peripheral nervous system.

The 12 pairs of cranial nerves arise from the brain and brain stem, the first is responsible for the olfactory system, mediating the sense of smell; the second, optic pair have the function correlated to vision, this nerve is a prolongation of the CNS. The control the muscles concerned with the movement of the eye oversee the third, fourth and sixth pair. The pair number five (trigeminal) has a widespread sensory and motor function, this nerve controls the muscles of the jaw and passing on sensation from the skin of the face and the mucosa of the mouth and nose. The compression of this nerve is a common cause of trigeminal neuralgia due to pressure sensory root by branches from the superior cerebellar artery. The seventh nerve is responsible for the nerve innervation on the superficial muscles of the face and neck, its susceptibility to injury is a notable aspect. The auditory system is connected to the eighth nerve, that mediates the senses not only of hearing but also the balance sense to the body. The ninth (glossopharyngeal) nerve is responsible for conveying sensibility from the pharynx and the back of the tongue; this nerve also has a minor motor function. The tenth (vagus) nerve has wide-ranging divisions and functions. The spinal part of the eleventh (accessory) nerve arises from cells in the accessory nucleus. The last nerve, 12th (hypoglossal) leaves the skull through the hypoglossal canal in the occipital bone to stream the intrinsic and extrinsic muscles of the tongue except for the palatoglossus. Most of the impulses felt by the human tongue travel in the lingual nerve even though there are receptor organs in the muscles of the tongue [23].

Figure 3 shows the location of the cranial nerves and the difference between efferent and afferent fibers with distinct colors [25].

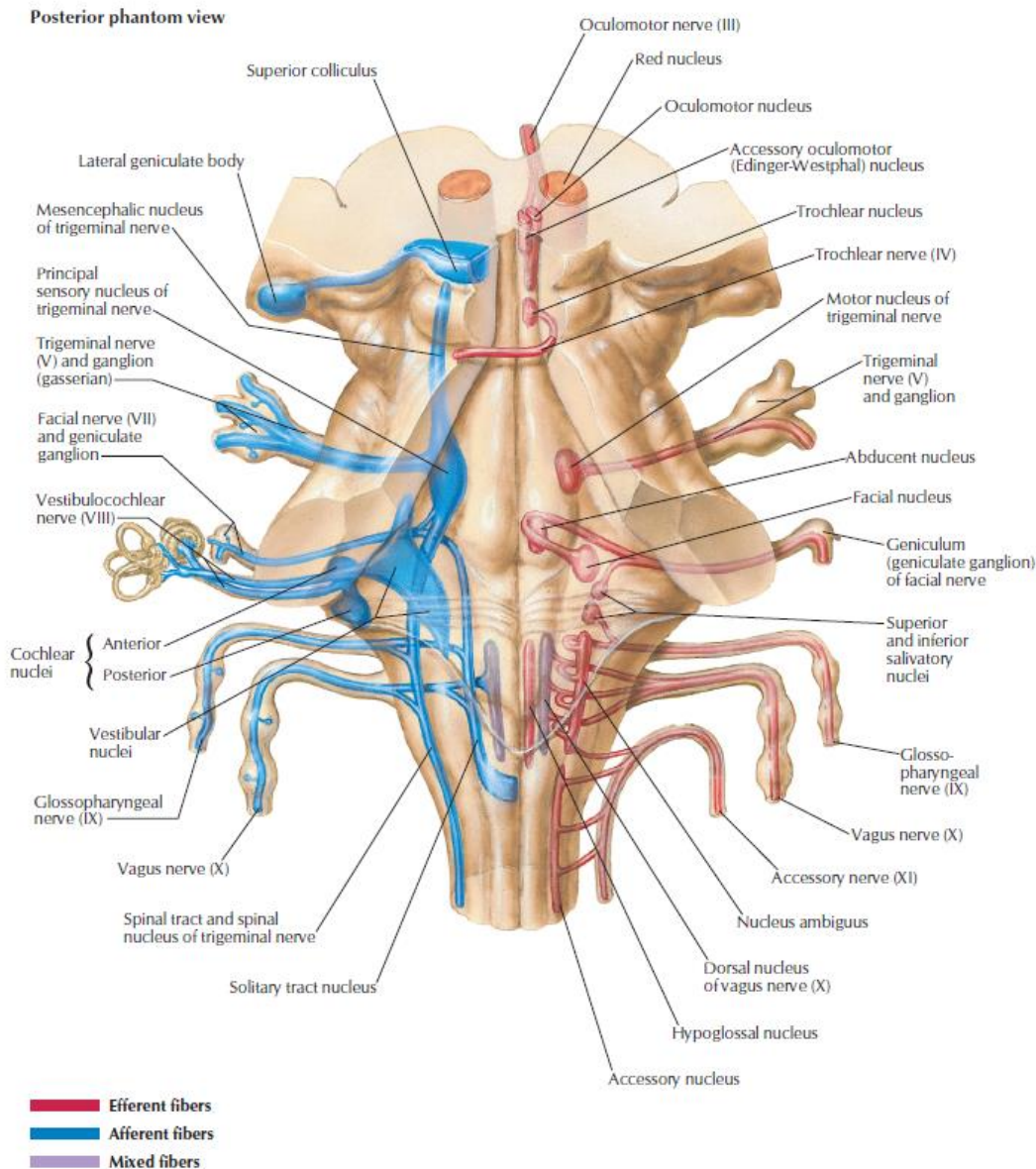


Figure 3 - Location of cranial nerves and the efferent and afferent fibers [25].

2.1.1 - Spinal Nerves - Structure and Nerve plexus

The main function of spinal nerves is to supply specific parts with sensory and motor function; they vary in diameter. From the 31 pairs of spinal nerves that arise from the spinal cord, there are 12 thoracics, eight cervical nerves, five lumbar, one coccygeal and five sacral. All the spinal nerves leave or arrive the cord by a ventral, largely motor, root, and a dorsal sensory root; this is represented in Figure 4.

All the spinal nerves are mixed nerves because they are molded from the mixture of nerve fibers from its dorsal and ventral roots. The afferent sensory root is the dorsal root, and he is responsible for carries sensory information to the brain. Thus, the ventral root is the efferent motor root; it carries motor information from the brain

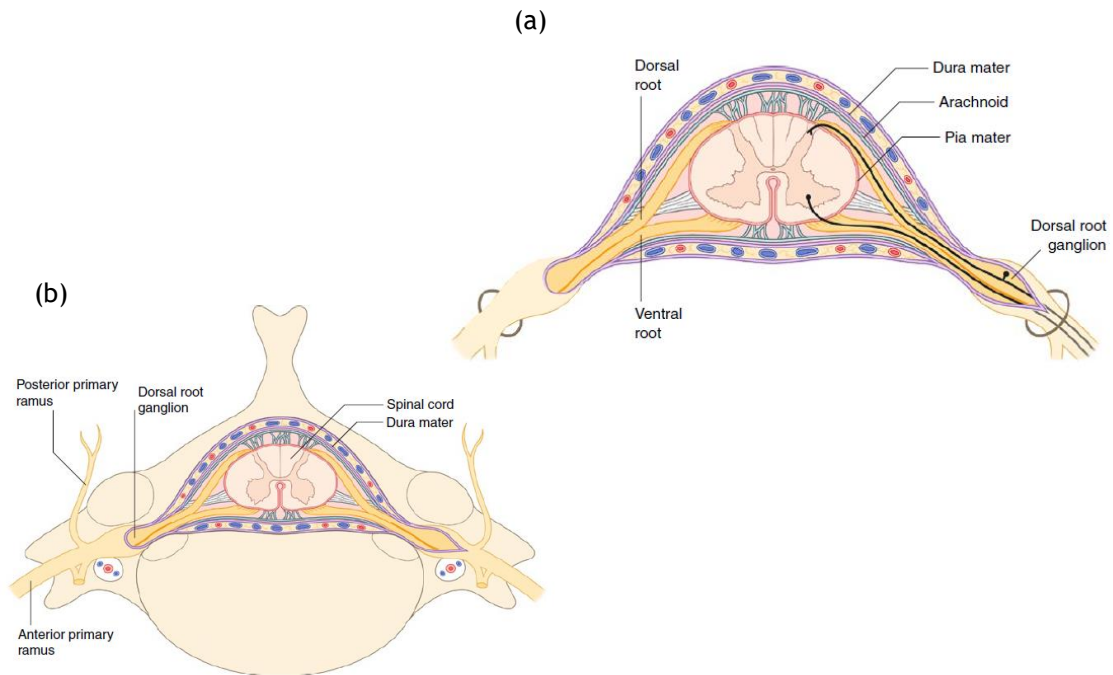


Figure 4 - Representative scheme of: (a) the source of the roots from the cord, their junction and the appearance of the nerve from the spinal cord. (b) The partition of the spinal nerve (cervical region) into anterior and posterior primary rami [23].

After exit, the intervertebral foramen the motor roots (anterior) and the sensor roots (posterior) merge and originate the spinal nerve according to the respective vertebral segment. The spinal nerve after exiting the spinal cord is divided into two different branches: a front branch called ramus anterior and a back branch called ramus posterior. The back branch does not form plexus, only the front branch. The spinal nerves can be divided into five different types according to the region of the spine: cervical, thoracic, lumbar, sacral and coccygeal.

The following figures Figure 5, Figure 6, Figure 7 and Figure 8 illustrate some of the most important peripheral nerves that derivate from the spinal nerves along all human body [23].

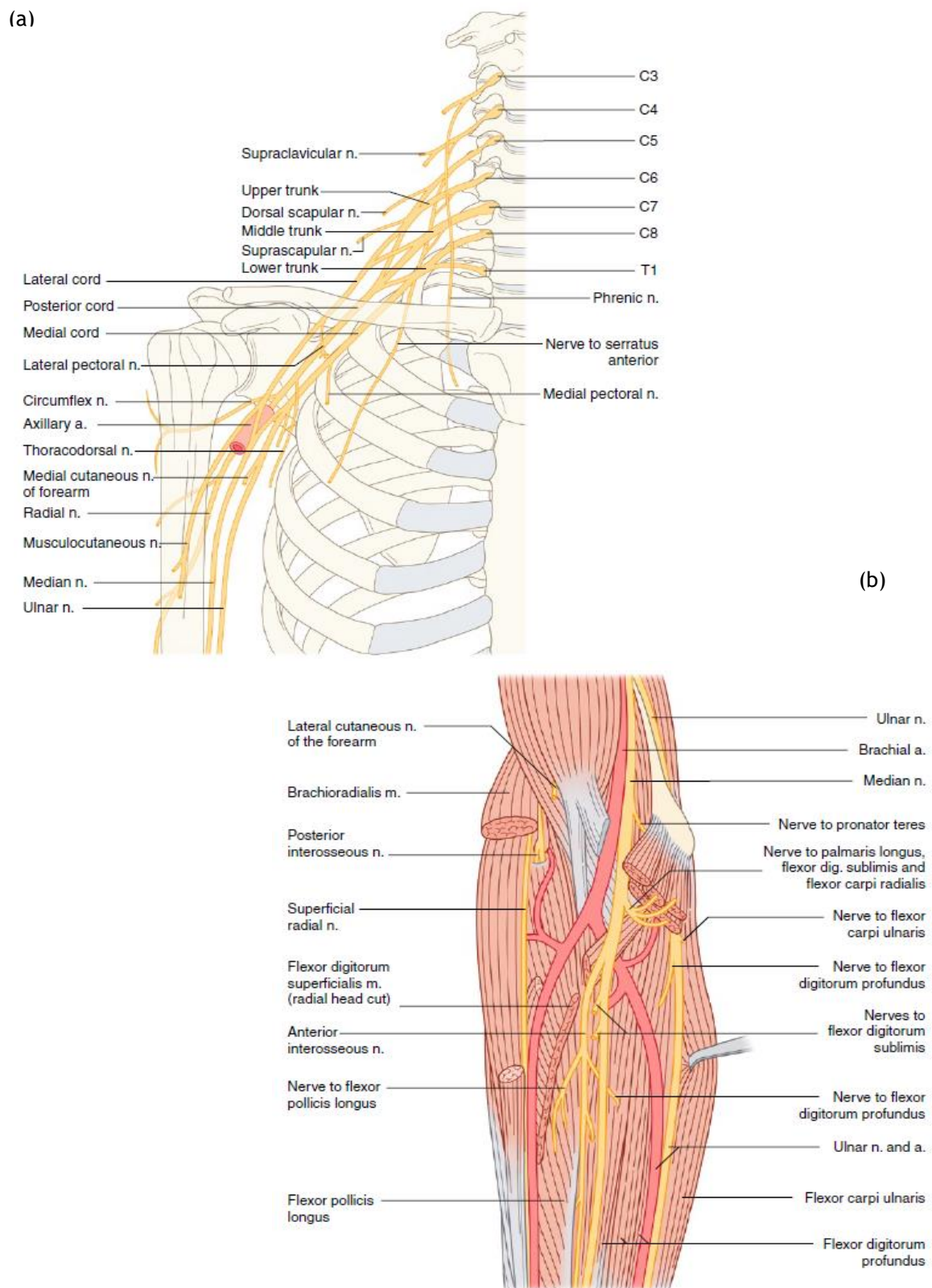


Figure 5 - (a) The right brachial plexus; (b) The anterior perspective of the right elbow [23].

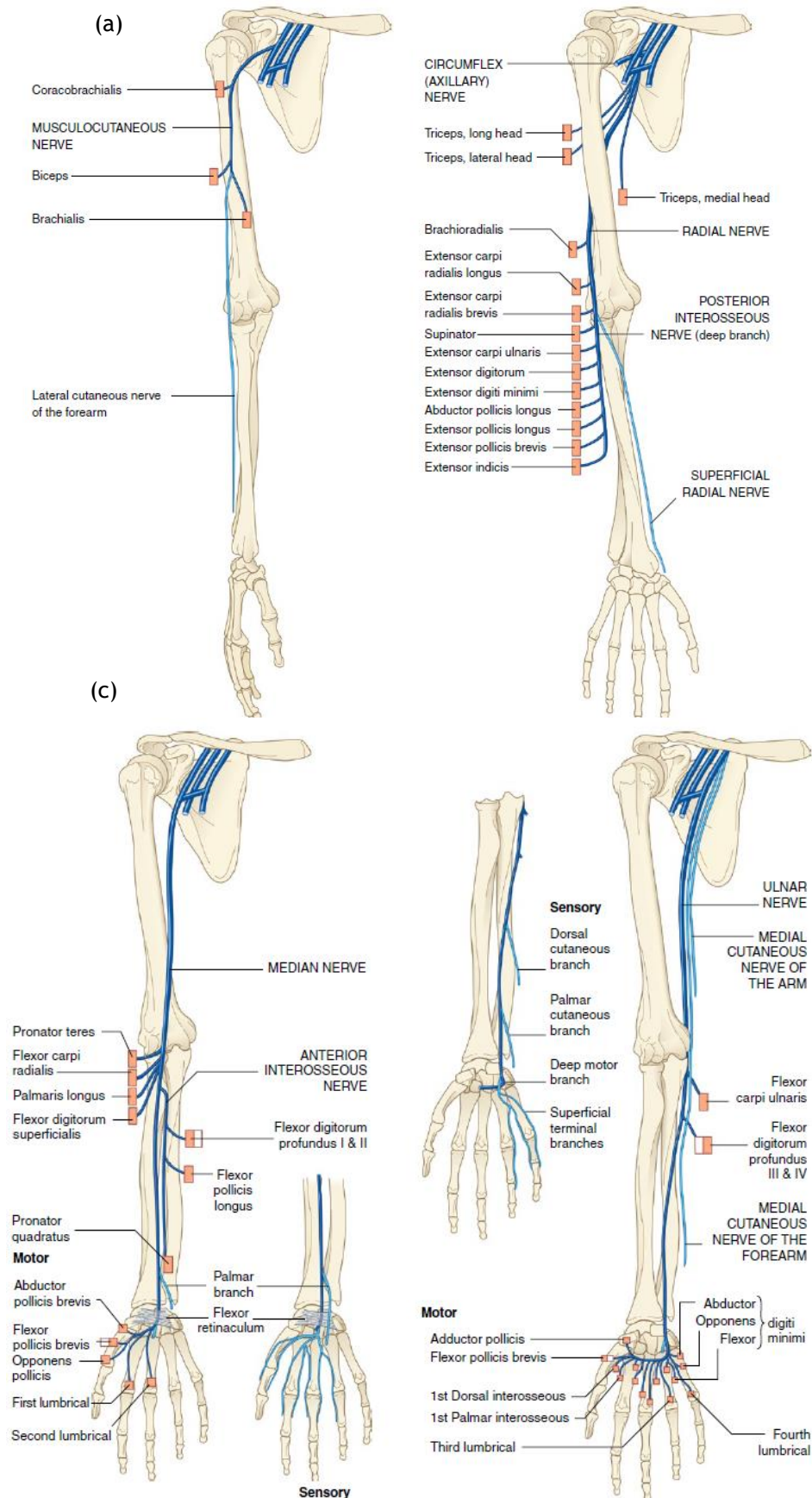


Figure 6 - The right : (a) musculocutaneous nerve; (b) radial nerve; (c) median nerve; (d) ulnar nerve [23].

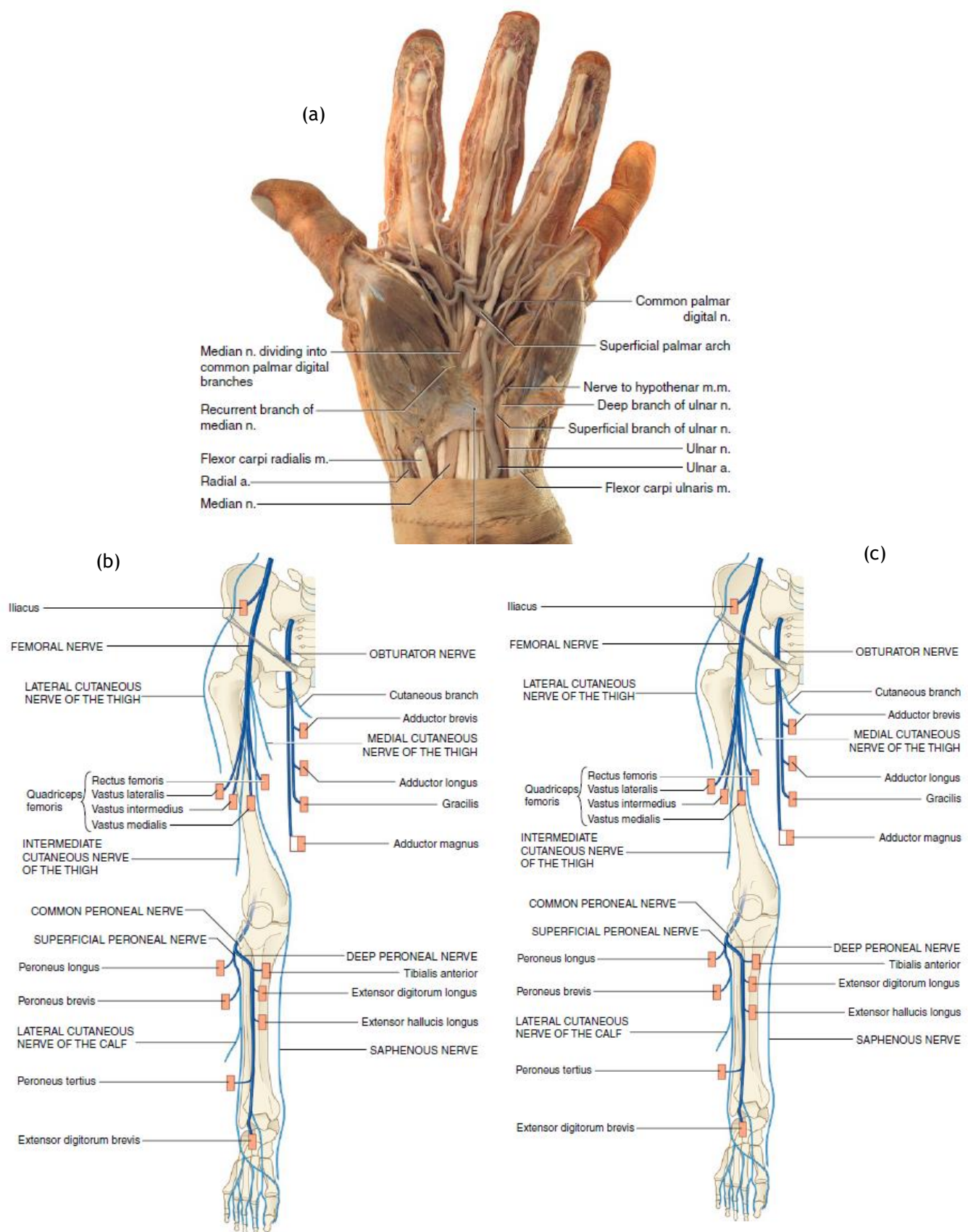


Figure 7 - (a) Median and ulnar nerves in the hand; (b) Nerves on the anterior aspect of the right lower limb; (c) nerves on the posterior perspective of the right limb [23].

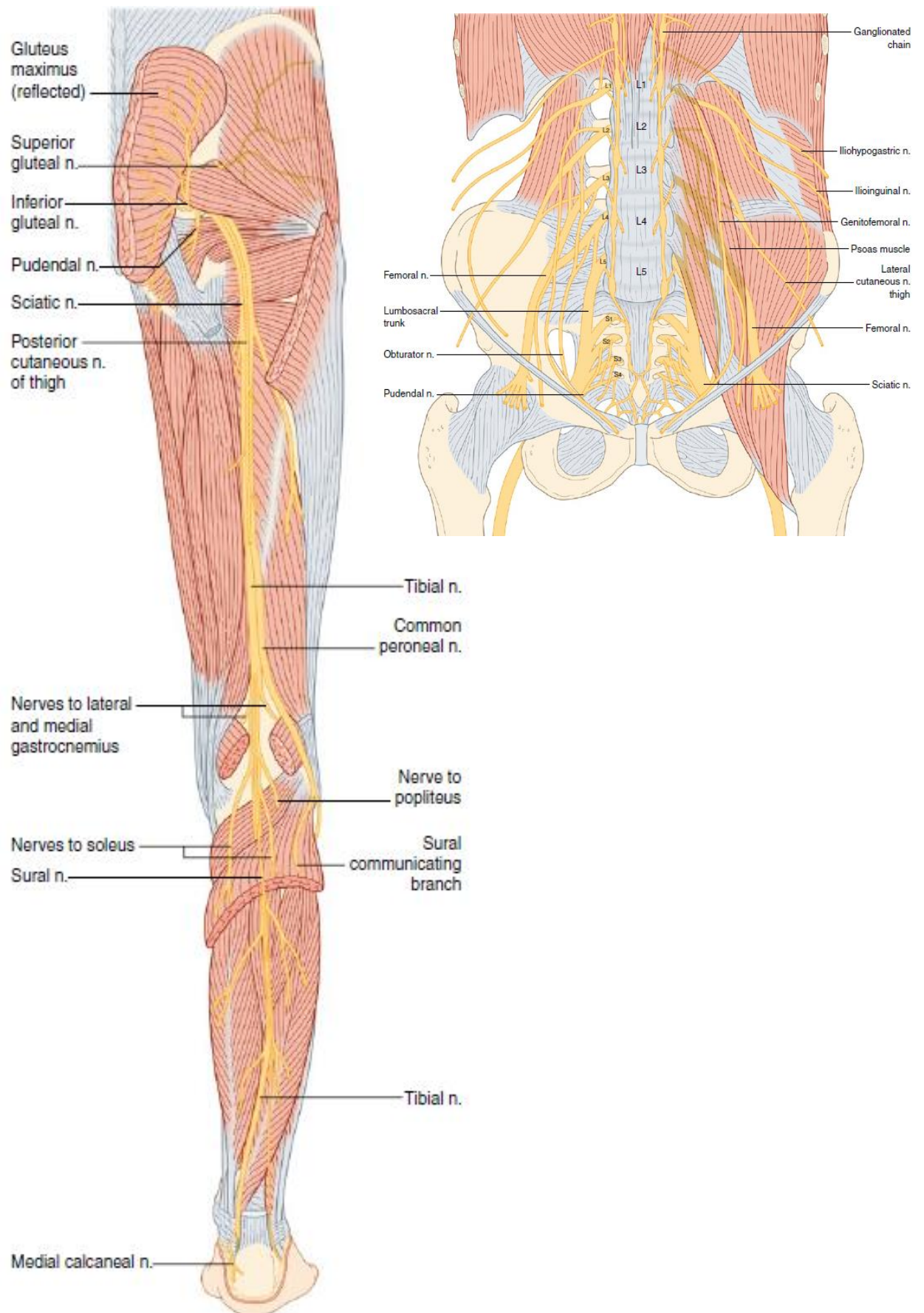


Figure 8 - Left - Right sciatic nerve and its main components in the lower limb; Right - Femoral and sacral plexuses and the ganglionated sympathetic chain [23].

The nerve plexus is a branching network (plexus) of intersecting nerves. The end of the branches of mixed sensory-motor nerves and the spinal nerve roots undergo alterations due to the development of myotomes. However, all segments of the spinal cord of that kind of nerves are arranged in the same way. The definition for myotome is the following: a group of muscles that are innervated by a unique spinal nerve. The posterior rami appearance stays the same, but the anterior rami suffer alterations, their primitive metameric division is preserved in the thoracic part, appendages emerge from the posterior part of the myotomes. Thus, the associated nerves, affected by these modifications, overlap, and divide, they form anastomoses and interlace to origin the plexus. Anastomoses are a network of channels that fork and recombine at various points (e.g., blood vessels). Figure 9 shows the different nerve plexuses and how they are organized [24].

There are different types of nerve plexuses, for example, the cervical and the sacrococcygeal plexus are composed only by simple nerve ramifications, with ascending and descending branches united with near branches. In the brachial plexus, sacral plexus and the lumbar plexus a new plexus is formed by the union of branches to originate common nerve trunks. The nerve plexuses are mainly founded in the top of extremities [23].

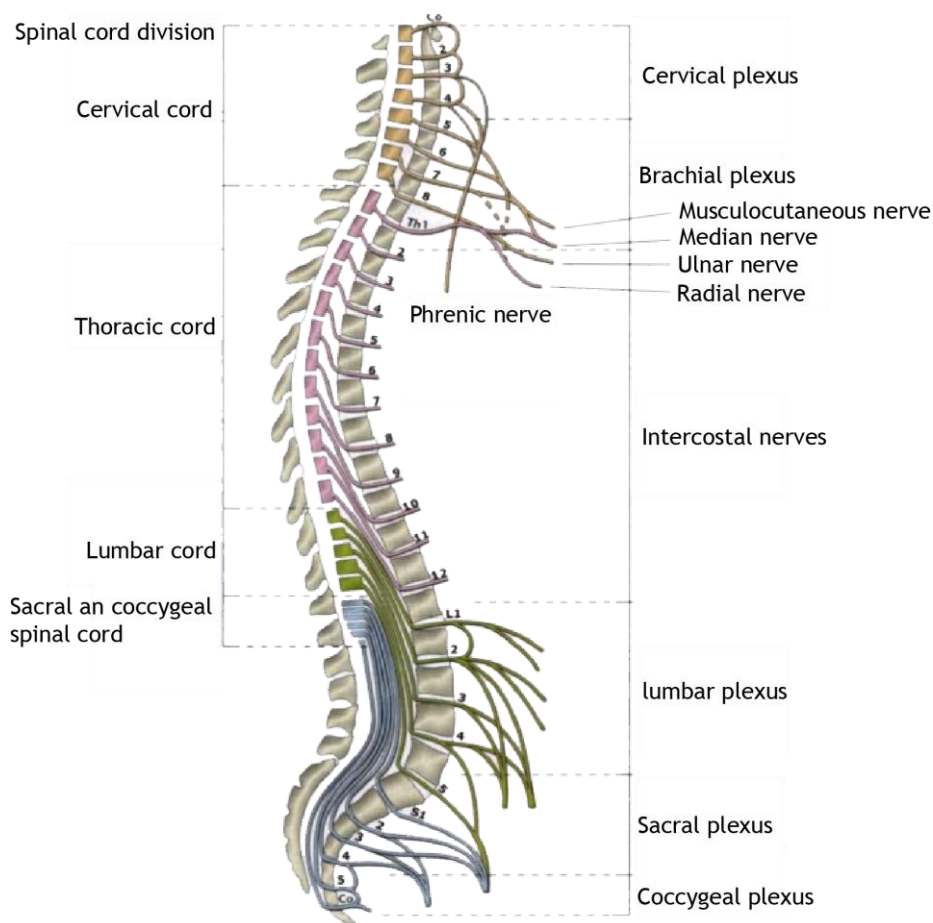


Figure 9 - Organization of the different nerve plexuses [24].

Another important subject is the nerve supply regions; there are three important regions: the sensory supply region (dermatomes), motor supply region (myotomes) and neurovisceral supply region.

Dermatomes are specific skin areas mostly supplied by a single spinal nerve of the posterior root with the interlineation of one or more peripheral nerves. The number of spinal cord segments is equal to the number of dermatomes. Topographically is possible to distinguish the different types of radicular innervation regions: poorly and better-supplied regions. In Figure 10 is represented a radicular topography of the human body showing the anterior and posterior surfaces [24], [26].

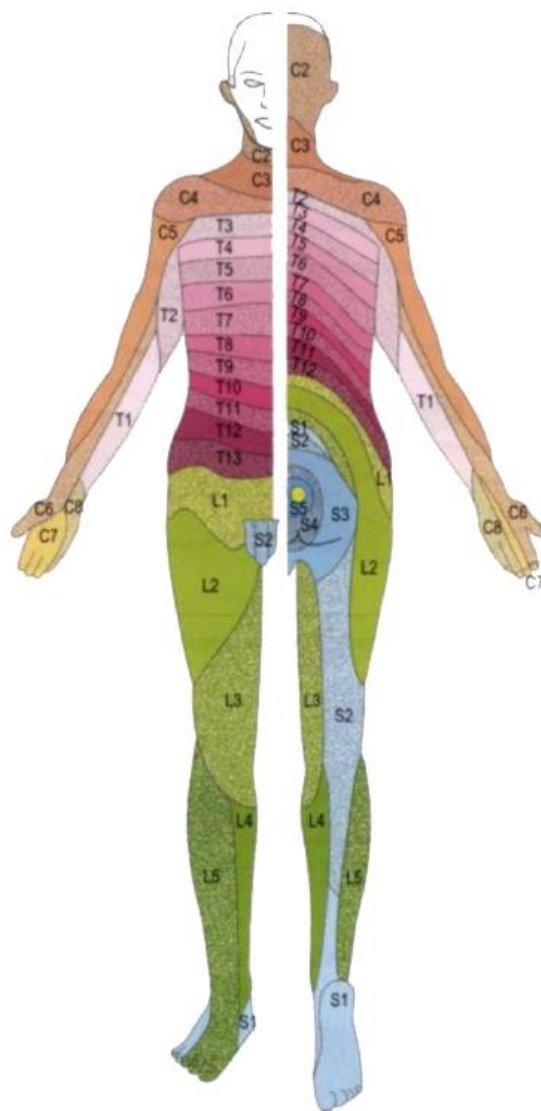


Figure 10 - Dermatomes of anterior and posterior surfaces in human body [24].

It is difficult in a single illustration to represent radicular innervation regions because they overlap each other. Every skin fragment has its nerve supply; however, it is frequently furnished by two adjacent nerves additionally. The radicular innervation regions are similar to lateral

strips pattern, at the trunk but when they are at legs and arms, they turn into longitudinal stripes. There are some important aspects between the dermatomes of anterior and posterior regions. There is more stretch in length and width on the dermatomes of anterior nerve branches.

The motor innervation (myotomes) follows a different pattern compared to dermatomes, is less schematic. Segmental nerves with their peripheral extensively ramified networks can innervate several muscles. Thus, a spinal cord segment can supply muscles from the same myotome through their plexus and peripheral ramifications. In most of the cases, the muscles originate from some myotomes are supplied by nerves of diverse segments. This can represent an advantage in case of an injury because the adjacent nerves can intervene.

Motor nerves ensure the neurovisceral supply, they leave the peripheral nerves and goes to the smooth muscles of the vessels and the sweat glands in the skin. It is the opposite to the sympathetic nerves [24], [26].

2.1.2 - Nerve fibers and nerve sheaths

There are two different kinds of cells in nerve tissue: Neurons and Glial cells. According to the most common definition, a neuron is the basic functional unit of the entire nervous system. It must do a multitude of tasks. The main functions of a neuron are receiving information from other cells, processes and transmits the information through chemical or electrical signals. However, the neuron must secure its auto-regulation.

Glial cells are responsible for physical support and to respond to an injury, this kind of cells regulate and coordinate the chemical composition that surrounds the cells. They participate not only in the blood-spinal-cord but also blood-brain-barrier, forming the myelin insulation and exchange metabolites with neurons. These cells have a diameter that varies between 4 and 135 μm [24].

From the cell body, there is the emersion of two different appendages: dendrites, that have the function of serving as afferent structures and conduct the nerve impulses centripetally. Thus, this structure increases the cell body surface.

The second appendage is the axon; this efferent structure conducts the nerve impulses centrifugally. In the terminal structure of the axon branch, there are small button-shaped hardenings, that are responsible for establishing contact with different cells or neurons. These small structures form a synapse with the adjacent cell membrane. Periakon is the nutrient supplier of the nerve cells. In Figure 11 is represented the typical structure of a neuron. There are two major criteria to classify the neurons: morphological or neurochemical.

The morphological subdivision takes into account the number and the form of the appendages. Multipolar neurons are the most common and the largest group of nerve cells; they are composed of several dendrites that arise from a polygonal cell body but only with one axon. In most of the cases, this type of neurons is associated with motor functions (motoneurons) or neurons of the visceral nervous system or Purkinje cells of the cerebellar cortex, or pyramidal cells prevenient of the cerebral cortex [23], [24].

On the other hand, bipolar neurons are composed of two nerve fibers. The first one arises from each end (pole) of the cell body. The contact with different neurons (synapse) is assured by one of the fibers. The other fiber is responsible for conducting the signals from the cell of

the axon. This type of neurons is founded in most of the cases in sensory organs (e.g., olfactory organs; inner ear).

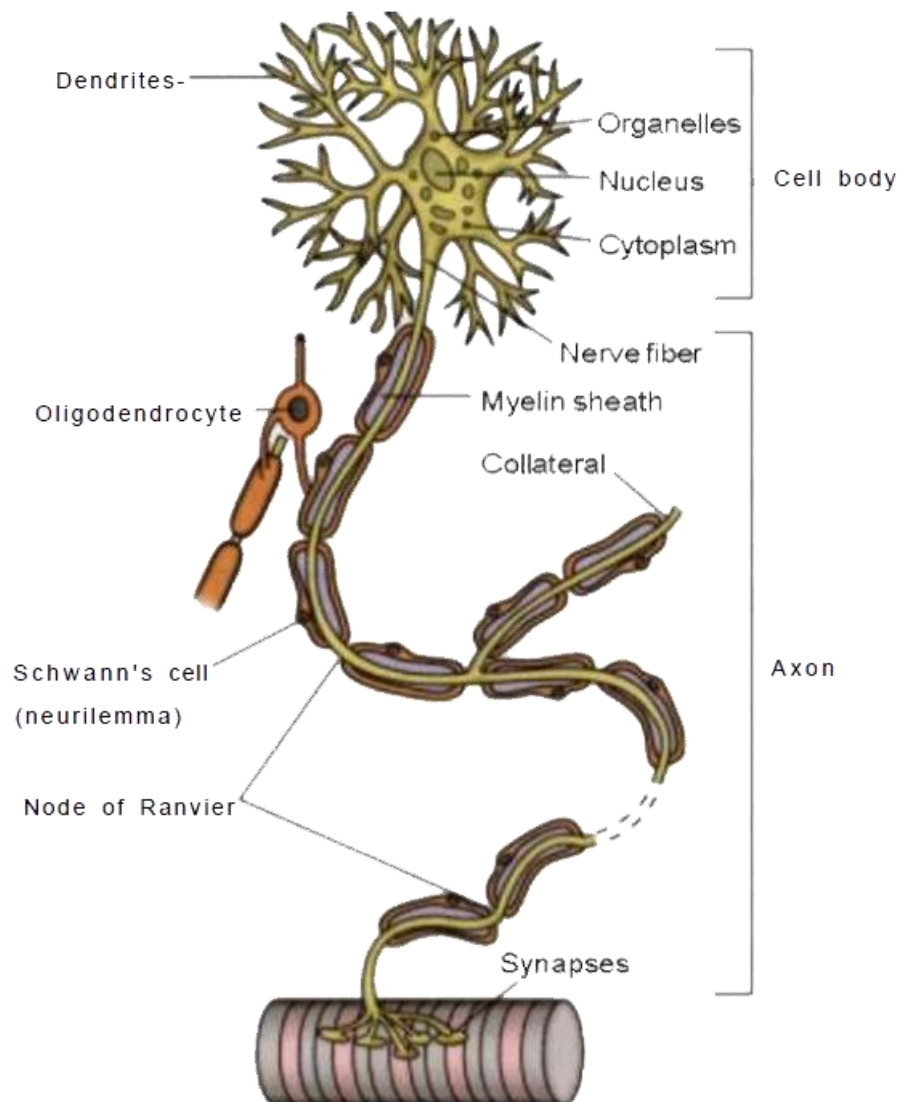


Figure 11 - Neuron Structure [24].

There are two major criteria to classify the neurons: morphological or neurochemical.

The morphological subdivision takes into account the number and the form of the appendages. Multipolar neurons are the most common and the largest group of nerve cells; they are composed of several dendrites that arise from a polygonal cell body but only with one axon. In most of the cases, this type of neurons is associated with motor functions (motoneurons) or neurons of the visceral nervous system or Purkinje cells of the cerebellar cortex, or pyramidal cells prevalent of the cerebral cortex.

On the other hand, bipolar neurons are composed of two nerve fibers. The first one arises from each end (pole) of the cell body. The contact with different neurons (synapse) is assured

by one of the fibers. The other fiber is responsible for conducting the signals from the cell of the axon. This type of neurons is founded in most of the cases in sensory organs (e.g., olfactory organs; inner ear).

The last type of neurons is pseudounipolar neurons; it is characterized by a big round cell body with one single appendage, that is divided into two branches after leaving the perikaryon. This neuron type can be found not only in the spinal ganglia of the posterior roots but also in sensory ganglia of some cranial nerves. Figure 12 illustrates the three different neurons according to morphological classification criteria.

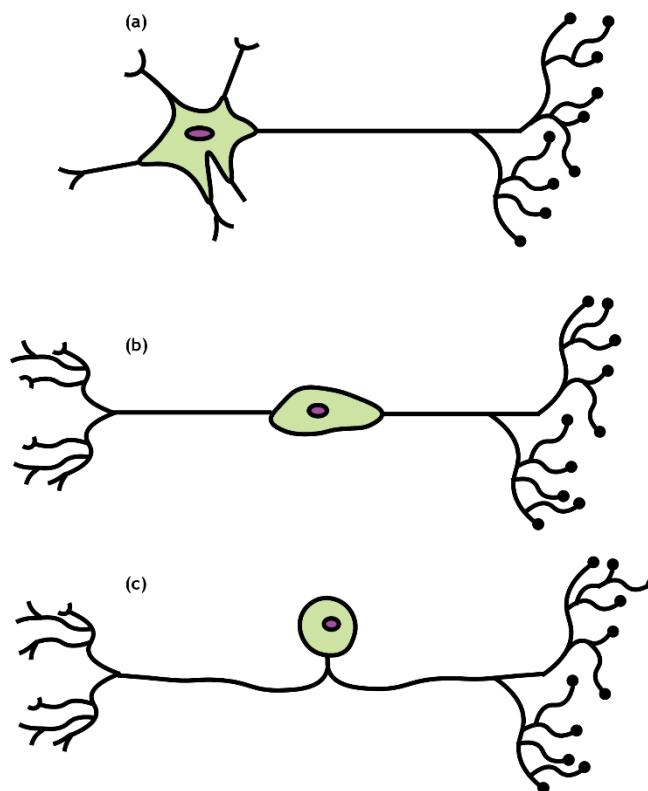


Figure 12 - Representation of the three different types of neurons: a) multipolar neuron; b) bipolar neuron; c) pseudounipolar neuron.

The type of transmitter and neurotransmitter released by the neurons is the main principle for the neurochemical division. This division consists of three different groups: one is, adrenergic, non-adrenergic or dopaminergic neurons, another is the cholinergic neurons, and the last consist in serotonergic neurons.

Excitability and conductibility are two different characteristics of the neurons protoplasm that is extremely developed and works together. Thus, the neurons can react to chemical or physical stimuli with the transmission of impulses that are called the action potential (excitability). The conductibility consists in conduct nervous impulses from one place to another.

Nerve fibers refer only to dendrites and the long axons. The peripheral nerves are composed of an assortment of nerve fibers involved by connective tissue, all the nerve fibers are

extremely organized structures of nerve cells, which every cell has not only an axon but also a Schwann's sheath [24].

In Figure 13 is represented a three-dimension schema of a nerve fiber, inside and outside.

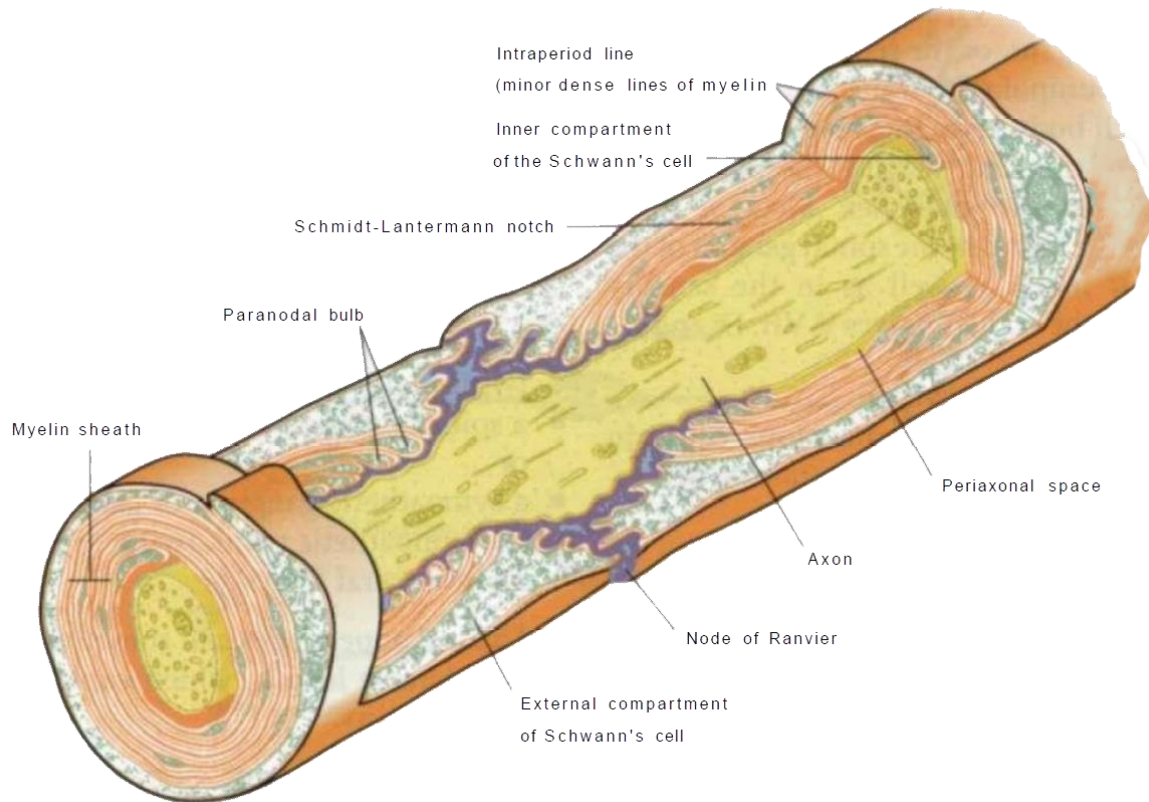


Figure 13 - 3D structure of a nerve fibre [24].

The myelin sheath is formed around peripheral nerves it is called neurilemma. Neurilemma is the Schwann's sheath around the axons, formed by Schwann's cells. The main function of this sheath is to accelerate the conduction of nerve impulses, without this, the nerve impulses are slowly. The nodes of Ranvier are formed by a short interval in the myelin sheath; these contact regions are involved in ions exchange. All the myelinated nerves have a protective sheath that is produced by its own Schwann's cells [27].

The myelin is mainly composed of lipids (70%) like phospholipids, cholesterol, glycolipids and only 30% of proteins. During the formation of the myelin sheath, the way it happens makes the external, and the internal plasmatic membrane layer of the Schwann's cells join. The myelination process amplifies not only the extension of nerve impulses but also save energy and space. The myelin prevents the outflow of ions by an isolation layer. The action potential is produced by depolarization of nerve membrane at specific sites (nodes of Ranvier). There is a difference between the central and the peripheral nervous system myelinization, in the central nervous system the nerve fibers can be myelinated only by one or the same oligodendrocyte [24].

Between the plasmatic membrane of the Schwann's cells on the peripheral nerves, there is a structure called endoneurium, consists an unshaped layer of connective tissue that spans the nodes and has functional importance, the thickness of this layer varies between 10-20 nm.

Nerve fibers are divided into different groups by their diameter/ thickness; the sheaths are included in this division, it is a morphological distinction. The separation between myelinated and unmyelinated fibers it is an important first division. The major differences between this different groups consist in the fact that unmyelinated fibers do not have a myelin sheath. However they have a simple Schwann's cell sheath, the neurilemma. Thus, a single Schwann's cell can enclose some axons so that several nerve fibers can be grouped. On the other hand, the myelinated fibers have only one Schwann's cell around a single neuron, with only a segment of myelin sheath but a thicker sheath [24]. According to Gasser and Erlanger (1925), there are three large divisions, that differ in structure and physiology of nerve fibers (Table 1):

- type A - myelinated fibers of the spinal and cranial nerves;
- type B - myelinated fibers of the autonomic/visceral nervous system;
- type C - unmyelinated fibers of the posterior roots of the spinal nerves and the sympathetic nervous system.

Another important aspect is the systematic classification of the nerve fibers, it is important to distinguish the nerves by the excitement they transmit (Figure 14). The motor efferent fibers and the afferent sensory fibers reversely lead the signal, the motor efferent fibers are responsible for conduct motor impulses from CNS (neurons of the spinal nerves in the grey matter of the spinal cord) to the striated skeletal muscles, it is descending conduction. This kind of fibers controls voluntary movements. The afferent sensory fibers in the inverse direction, from the skin receptors (exteroceptors or proprioceptors in the deep) to CNS, it is ascending conduction. The neurons that control these fibers are in posterior sensory root (radix dorsalis) in the spinal ganglia or the sensory ganglia of the cranial nerves, their function depends on the fiber thickness. The thickest conducts the tactile stimuli and proprioceptive, the medium temperature sensation and the thinnest pain impulses or somatic discomfort. All the muscles have motor and sensory fibers. Thus, the automatic coordinated interaction of agonist and antagonist fibers during muscle contractions needs innervation from the sensory nerve fibers [24].

Sympathetic or efferent neurovisceral fibers control the secretion of the glands, visceral function, and the nutritional state of the tissue, they innervate the smooth muscles of the visceral organs, body hair, and vessels. The neurons responsible for these fibers are in the sympathetic ganglia and spinal cord [24].

Some afferent impulses that do not belong to the sympathetic system arising from the visceral organs or the vessel walls attached to sympathetic or peripheral nerves in the direction of the CNS [23], [24].

Table 1 - Gasser and Erlanger classification according to structure and physiology of nerve fibers [24].

		Myelinated					Un- myelinated
type		A				B	C
subtype		A α	A β	A γ	A δ		
diameter		12-20 μm	5-12 μm	2-10 μm	2-7 μm	<3 μ	0.25-1.5 μm
Conductivity speed		70-120 m/s	30-120 m/s	10-45 m/s	12-30 m/s	3-14 m/s	0.4-2 m/s
Nervous system		CNS			Sympathetic and parasympathetic	Mainly sympathetic	
functions	CS E	α -motor neuron		γ -motor neuron		Preganglionic (VNS)	Postganglionic (VNS)
	CS A	Deep sensitivity Muscle spindles and Golgi-tendon organ	Tactile (touch) stimuli, pressure, vibration		Tactile (touch) stimuli, temperature, fast pain conductivity, visceral and vascular stimuli		Visceral stimuli, slow pain conductivity, Temperature perception

¹CSA- common somatic afferents; CSE - common somatic efferents; VNS - visceral (autonomic) nervous system

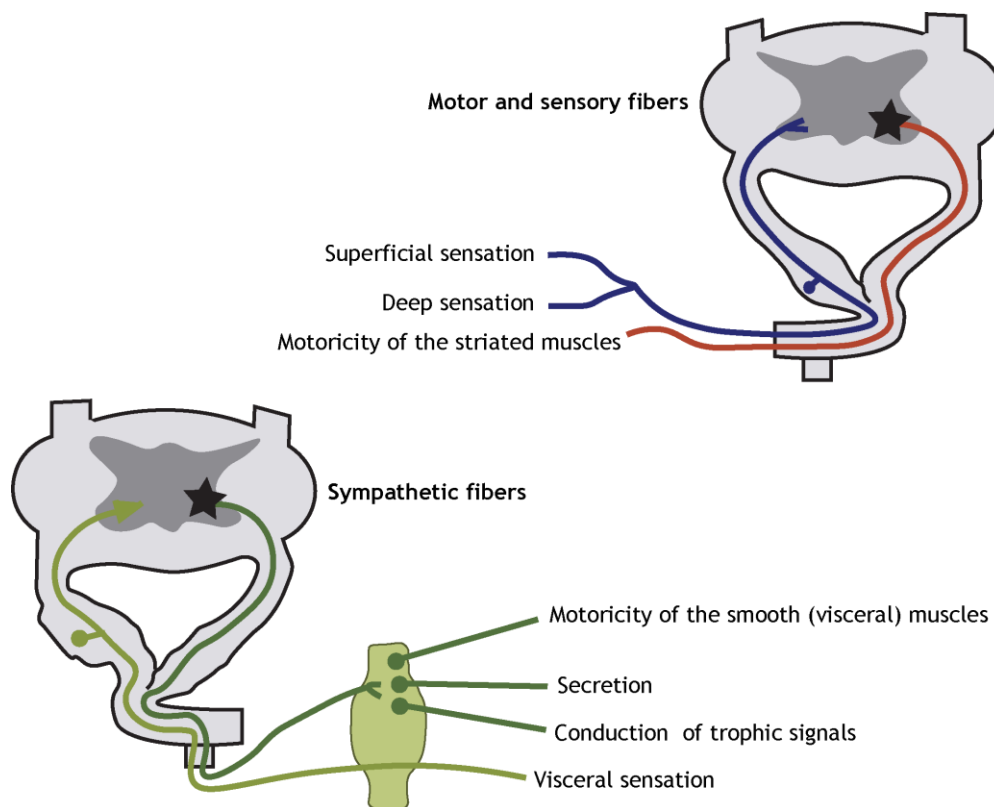


Figure 14 - Different types of nerve fibres.

2.1.3 - Peripheral nerve, Connective tissue structure and vessel supply on peripheral nervous system

In the nervous system, the fibers are found in bundles, not as a single fiber. Thus, the junction of several fiber bundles originates the nerve stem. The nerve fiber bundles are a basic functional unit called fascicle. A connective tissue layer environs the nerve fibers with a variable thickness. The different types of the connective tissue of peripheral nerves can be classified according to its topography and anatomy. Thus, there are three different types: endoneurium, perineurium and epineurium (Figure 15) [24], [28].

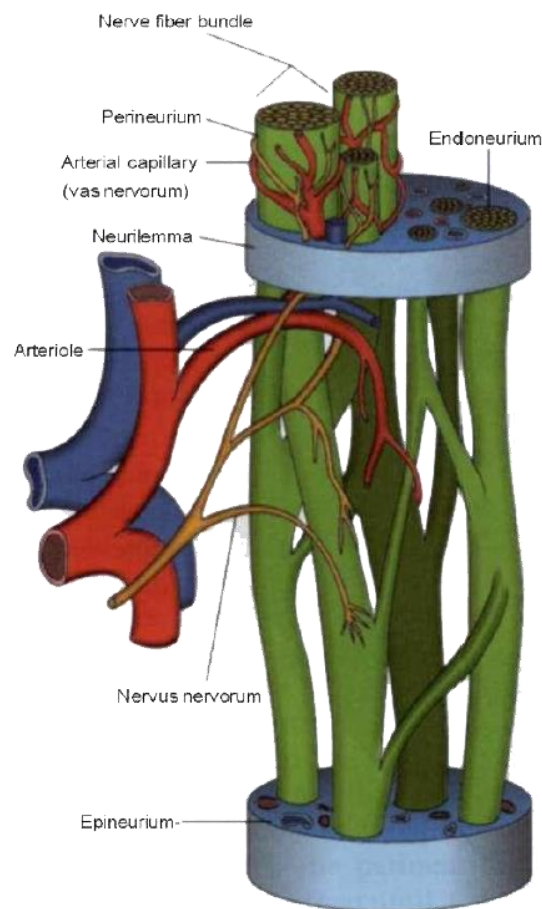


Figure 15 - Schematic of the composition of a peripheral nerve [24].

The endoneurium is the intrafascicular connective tissue and the primary fascicle, bundling a group of nerve fibers. Thus, endoneurium is a loose connective tissue, an extension of the basal membrane that wrappers the Schwann's cells of the nerve fibers. It is composed of compressed fibroblasts and thick collagen fibrils (30-50 nm) that are disseminated over the longitudinal direction of fascicles. Inside the endoneurial space are red blood cells, macrophages, and capillary vessels. The main function of this tubular structure is to protect the nutrition of the nerve fibers. This structure is responsible not only for protecting the axons from the traction that can occur because of the mainly longitudinal orientation of the collagen

fibrils but also have an important role in maintaining constant the light positive pressure in the fluids of the endoneurial space. Without lymph vessels, in the endoneurium, the conduction of nerve impulses is not affected by the pressure variations in the axoplasm. Greatest of endoneurium portion is in skin nerves because nerves run closely under skin's surface [24], [28].

The second structure is the perineurium, it is the perifascicular connective tissue, a thin multi-layered coat, that contains numerous primary fascicles grouped into a secondary fascicle. The perineurium outer coat is made of connective tissue concentric layers with dense collagen components. This structure is composed of fibers arranged in a longitudinal and ring-shaped way, that fibers are tightly twisted and coarse. The perineurium has a few fibroblasts and macrophages, forming a singular feature composed of a structure of fibroblasts and glide layers. The most common is to find seven or eight layers in nerve trunks. However, in some mammals, the layers number can reach fifteen. The densely packed fibroblasts layers that relate to each other cell to cell by tight junctions which avoid the passage of substances among layers are the perineural cells. The diameter of the collagen fibrils varies between 40 nm to 80 nm, but the perineurium had only 1 μm of thickness. The perineurium has three major functions: protection the endoneurium and its contents; diffusion barrier, against certain substances that can interfere in the intrafascicular environment and the last function is to create a barrier against external mechanical forces. Perineural scratches can inhibit the conductivity of peripheral nerves for a considerable period when a sciatic nerve suffers a contusion the conductivity is disturbed at least four months at the injured site. If the perineurium is an effective diffusion barrier for external substances to the internal fascicle, when this structure suffers a contusion, proteins may enter and can reach the endoneurium [29]. Thus, nerve fibers can be altered for a long time and sometimes long distances. The perineurium is strong against ischemia, they can hold for 24 hours without lesions. On the surgical field, nerve repair sutures can only be completed in the perifascicular tissue, as it possesses enough stability to hold them. Otherwise it would break. Due to his strong structure of collagen and minor amounts of elastin the perineurium is very resistant to traction forces. According to traction tests, the perineurium It is a very sturdy structure that did not tear until it reached an intrafascicular pressure of 300-750 mmHg ensuring good protection to the nerve fibers from mechanical strains. However, it tears, due to a positive intrafascicular pressure and its content leaks at the injured site. Thus, around this perineural "gap," focal demyelination of the underlying nerve fibers can occur [24].

The epineurium consists of the interfascicular connective tissue that bundles numerous secondary fascicles into a peripheral nerve trunk. It is composed of dura mater this peripheral branch. This structure not only divides the secondary fascicles from each other but also forms an external layer around all fascicles. It is stronger than the other connective tissues and it is composed of 60- 100 nm collagen fibrils. The most common orientation of the collagen bundles is longitudinal like the other structures mentioned before but also a little oblique. The epineurium is mainly composed of a high number of collagen fibers that increases the mechanical resistance of the nerves, between these fibers there are different types of cells like fat cells, fibroblast, and red blood cells. The external layer of epineurium has blood vessels. The main functions of this structure are envelopes the fascicle and to protect and cushions. However, the epineurium has: nerves (nervi nervorum) from the sensory and sympathetic systems, originated in the perivascular plexus and in the actual nerve fibres; Vessels (vasa nervorum) located between the fascicles in the connective tissue are responsible

for the peripheral nerve nutrition, branches go off from large arteries and enter in the epineurium, then ramify in different directions narrowing in small vessels in perineurium; mobility, responsible for the movement of the arms and legs due to ability of fascicles to glide through the epineurium internum. This adjustability is very significant when nerves bend at an acute angle. The peripheral nerves are the tissue with the larger movement margin in comparison with the other tissues on their environment [24], [28].

The last structure that surrounds the peripheral nerves is the mesoneurium; it is a loose connective tissue. Blood Vessels can enter in the mesoneurium in different places. According to different researches, this structure does not have a specific function, but for example, in the case of injection, this structure allows to glide the peripheral nerve laterally. Although this structure does not yet have a specific function determined, mechanically it plays a significant role.

In Figure 16 is represented a schematic division of the connective tissue on the peripheral nerve [24].

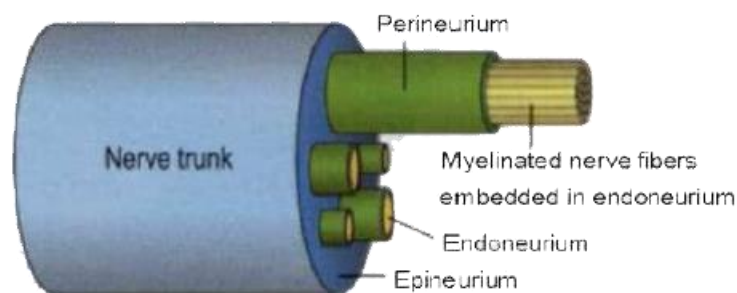


Figure 16 - Simplified representation of the different connective tissues on the peripheral nerve [24].

A large part of peripheral nerve composition is occupied by the perifascicular and interfascicular connective tissue. However, the small roots in the spinal cord does not have connective tissue. The size of the fascicles influences the amount of connective tissue; the axillary nerve has the highest percentage of connective tissue (~90%), the ulnar nerve presents an approximate percentage of 60% and the median nerve 50%. Thus, the peripheral nerves present different mechanical strains according to his location and function, Higher connective tissue percentage means higher strain.

To ensure all the nutrient supply for the peripheral nervous system, there is substantial variability in vessels networks. In most of the cases, the arteries branches to the nerves, originate in the vessel trunks located close. Thus, multiple blood sources can supply a nerve segment. Before sending smaller branches to the epineurium, all artery primarily branches off into an ascending and descending branch. The capillary frame on the fascicles is formed by the venues and the smallest arterioles that move transversely through the perineurium to the endoneurium. However, the endoneurium only has capillary vessels.

The nervous connective tissue contains arcades of longitudinal connection vessels at irregular intervals, due to nerve flexibility they pass through the mesoneurium, curving and winding. Thus, it gives to the nerve a certain degree of freedom/mobility. There are several patterns that describe the course followed by arteries: In one hand, If the primitive artery runs

parallel to the nerve, the supply arteries can divide into transverse or oblique, ascending and descending branches (Figure 17 a) or they can emit one or more branches by moving directly along the nerve (Figure 17 b). On the other hand, the primitive artery can directly cross the nerve. Thus, new branches below or above this intersection can move back toward the nerve (Figure 17 c) [24].

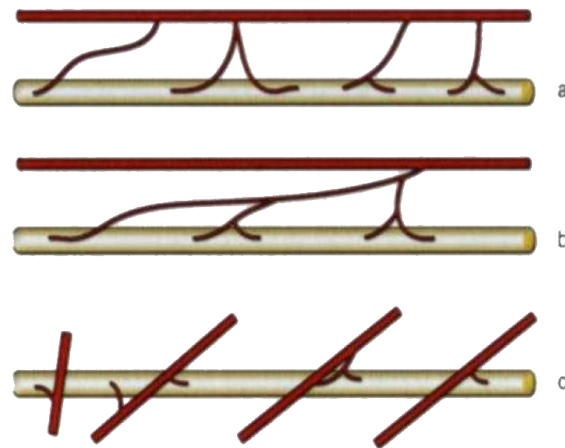


Figure 17 - Spatial distribution and disposition of blood vessels in nerves (vasa nervorum).

Inside the nerves, on epineurium, there are a big number of smaller arteries and veins responsible for intrinsic supply, most of them on a longitudinal distribution and extensively ramified. When inside the nerve, the vessels can go directly to epineurial or perineurial longitudinal arcades or move with interlineation of cross connections. The large arterioles do not change their diameter on perineurium. Inside the endoneurium, the blood supply is ensured by capillary vessels positioned transversely, longitudinally or obliquely. Several anastomoses connect them to perineurium. During an injury empty parts of nerve capillary fill up. Thus, a massive endoneurial blood supply protects the nerves from ischemia; normally the blood flow does not have only one direction, it can be reversed in any place. In Figure 18 is represented a simplified schema of the complex intraneural microvasculature structure [24].

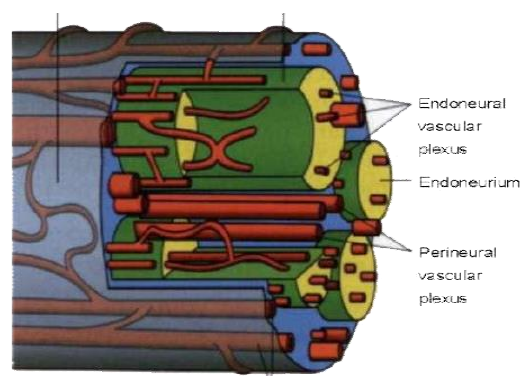


Figure 18 - Intraneural microvasculature structure [24].

2.2 - Types of injuries and accidents

The traumatic nerve damage can be classified by two different major systems: Seddon system or Sunderland system. The first system, Seddon system (1972), divides the lesions in only three different patterns: Neurapraxia; axonotmesis and Neurotmesis. The second system is more extensive and comprehensive in the damage scale. Thus, Sunderland (1968) divides the nerves damages into five different scales. Although the classification systems are different, they are intrinsically linked and analyze the same anatomical factors however the Sunderland system becomes more accurate due to having a greater division scale of damages [30].

The first state of Seddon system is neurapraxia, which consists of a nerve concussion (e.g., when dysfunction of a peripheral nerve without nerve fiber degeneration occurs). This type of nerve damage recovers in a few days, as some manual therapies can speed up the recovery process. The second is axonotmesis, which happens when a nerve contusion occurs with the damage of several axons, but the continuity of the nerve sheaths remains unbroken. The last is Neurotmesis, which is the most serious of all. It is a nerve injury with the severance of nerve sheaths and axons, in most of the cases the continuity is compromised. Neurotmesis need surgical intervention; it cannot recover naturally [24].

According to Sunderland classification, five different codes are increasing the number with the severity of the injury. This classification takes into account the following affected areas: axon continuity, axon transport, continuity of the nerve fibers, perineurium, fascicles and the continuity of the nerve trunks. There are different causes of nerve damages, so the effects are not uniform on the tissue. In this scale the grade 1 corresponds to neurapraxia; the grade 2 to axonotmesis; the grade 3 is between axonotmesis and neurotmesis, so it has a variable reversibility, due to several factors as the size of lesion or the severity level of the interfascicular damage; the grade 4 and 5 corresponds to neurotmesis and refers to cases without natural improvement, in grade 4 there are severe intraneural disorganization. Grade 5 occurs the total loss of nerve continuity. Table 2 shows a schematic correspondence between the two classification systems [24].

Table 2 - Schematic correspondence between Sunderland's and Seddon's classification systems [24].

Classification		Seddon's		
		Neurapraxia	Axonotmesis	Neurotmesis
Sunderland's	1 st			
	2 nd			
	3 rd			
	4 th			
	5 th			

As previously mentioned there are two different systems to classify the effect of the injury on the nerve, but it is also important to know how this injury occurred and classified them. According to Horsley (1899), the most common classification for nerve injuries consists in four different classes: first division, second contusion, third compression and the fourth is stretching [31]. When occurs the complete division, and there is nerve separation with severe

intraneural damages. Several traumatic lesions causes demyelination and/or interruption of the axon continuity. The demyelinic fibers are less prone to damage, due to the small diameter when compared to the myelinic nerve fibers. A contusion is a localized injury on the nerve (e.g., when something hit the shin).

When a nerve suffers a lesion through compression, there are several factors to take into account, as the kind of strength of the pressure or the duration of the incident. The space between the fascicles due to loose connective tissue offers some protection. The relative position between the fibers influences the protection; the deep fibers are protected by superficial fibers. The nerve compression affects in the first place the myelin sheath. Thus, continuous compression on the nerve can reduce or eliminate them. However, the axons also suffer with the compression in a second place, and they can be seriously damaged, then starts the Wallerian degeneration above the lesion location. The compression in nerves origins a combination of changes, not need to be “all or nothing”. There are cases from neuropraxia to neurotmesis according to the severity degree suffered by the fascicles. Nerve compression leads to an interruption of conductivity in the injury site, unleashing a complex vicious cycle where occur ischemia, transudation, internal pressure increases, lymph, and venous flow reduce, break of arterial blood supply and finally occurs nerve damage.

The stretching injuries nowadays are increasing with violent car accidents. In most of the cases, the brachial plexus is affected [32]. Traction lesions cause much more extensive damage to nerve-trunk that other types of injuries [33], [34]. Figure 19 represents some examples of nerve fiber lesions.

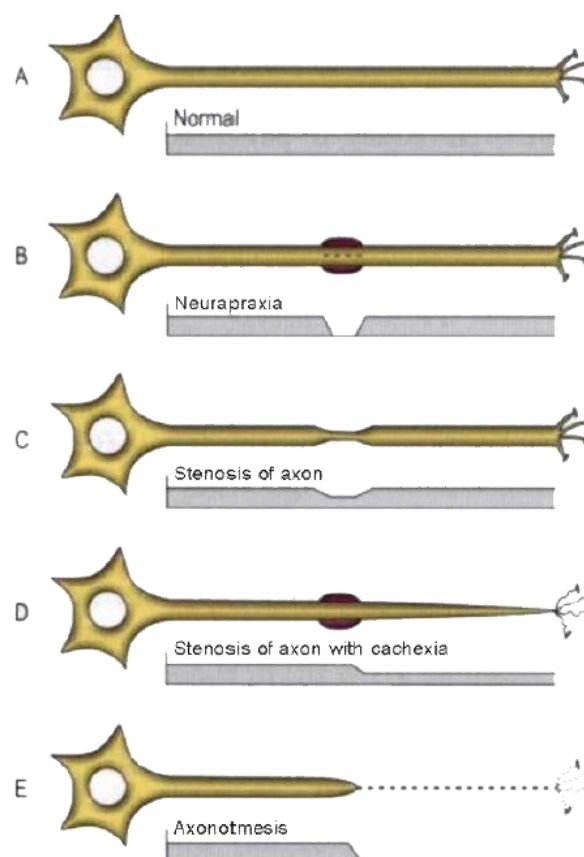


Figure 19 - Schematic examples of nerve fibre lesions and conductivity speed graphs [24].

The symptomology is influenced by several factors such as pain occurrence during rest and the increasing of pain or the different intensity of pain due to certain movements or positions. The severity of affected motor or sensory fibers influences the symptoms, in acute forms exists much pain, and malfunctions simply recognize it. The junction of pain and paraesthesia may indicate chronic nerve damage [24], [35].

2.3 - Peripheral nerve regeneration and therapy

Nowadays, the regeneration of damaged or defective peripheral nerves stills a big challenge for neuroscience it is a large-scale problem that affects millions of people lives annually [11], [36]. Spontaneous regeneration may occur in short nerve gaps, with or without the help of manual therapy, but in large gaps, the chirurgical intervention is needed to repair the damaged nerve. The use of biocompatible tissue-engineered scaffolds is an emerging research topic due to biomaterials potential and capabilities to solve these problems [11].

Independent of the type of treatment, a reduction in pain does not certainly mean an improvement has happened in nerve recovery because pain can disappear even if sensory fibers are injured. There are two different approaches to repair a large injury in the PNS: end-to-end suturing and bridging. The end-to-end suturing or the direct nerve repair is only used when the patient has a small nerve gap (<5 mm) and require the tension-free between distal and proximal nerve end. It is the preferred method, but the nerves need to be perfectly aligned and repaired for ideal regeneration. Bridging nerve gaps of a critical length (>3cm) with an autologous nerve graft (autograft) is the best option when it is not possible to suture the injury. Autografts still the gold standard for the last 50 years. However, this method has some problems and disadvantages, is far from ideal and is frequently unsatisfactory due to several factors such as these grafts are primarily sensory nerves due to the unavailability of motor nerves, so there are anatomical differences; it is necessary to scarify a health nerve on a second chirurgical intervention; the donor site morbidity and other factors that influence the success rate of this procedure. Only 50 percent have a successful recovery with this technique [12]-[15]. The use of allografts has some disadvantages too; the patient needs an extensive immune suppression over 18 months after the implantation becoming more susceptible to infections.

A viable alternative to overcome the nerve grafts difficulties consists of the Interposition of nerve scaffold whether artificial acellular conduit or a cellular allograft [15]. The scaffold should contain the following requirements: (1) biocompatibility with the tissues, (2) nonimmunogenicity and nontoxicity (3) biodegradability at an optimum rate, (4) appropriate porosity and morphology and (5) ideal mechanical properties [16].

The first and major alternative, clinically approved, is the use of hollow nerve guidance conduits (NGCs). There are several advantages with the use of NGCs for nerve repair such as the elimination of donor site morbidity, the reduction of neuroma, restricted myofibroblast infiltration and enables the accumulation of an to elevate concentration of neurotrophic factors. The NGCs have an important role in the guide to the distal targets the damaged nerves; they create a controlled microenvironment that allows the nerve fibers regeneration. The translated NGCS are mostly made from synthetic materials such as polylactide-caprolactone (PLCL), poly-glycolic acid (PGA), various combinations of the PGA or PLCL, or from collagen or chitosan founded on nature. The regenerative process follows a sequence of five distinct

phases: (i) the fluid phase; (ii) the matrix phase; (iii) the cellular migration phase; (iv) the axonal phase; and (v) the myelination phase (Figure 20) [11].

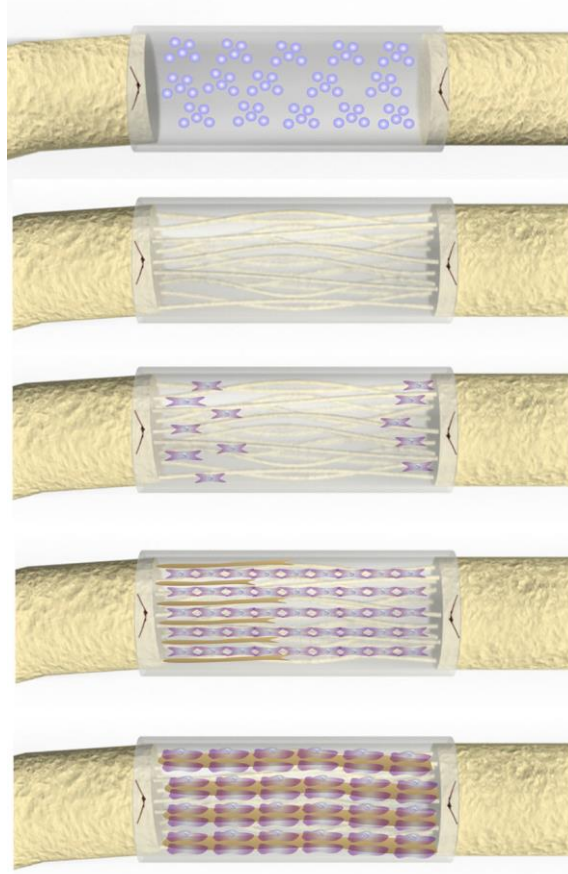


Figure 20 - Regenerative process sequence using an NGC hollow [11].

2.4 - Mechanical properties of peripheral nerves

The peripheral nerves trunks and their sheaths are considered viscoelastic tissues with exclusive mechanical features. The study of tensile strength including elastic limit and mechanical failure is very important to improve medical surgeries [37], [38].

Daily, nerves suffer various normal mechanical stresses executed by posture and movement. The stress can be defined as a force divided by the area where it acts [39]. The nerve can suffer tensile, compressive, or shear stress or as a mixture of stresses (Figure 21) [38]. The longitudinal or transverse stress can be produced by tensile stress which is applied either parallel or perpendicular to the length of the nerve. During the joint motion, the nerve accommodates the stress by elongating and gliding, through this process the nerve is exposed under tensile stress. The strain, characteristically expressed as percent elongation is caused by longitudinal tensile stress origins deformation or alteration in nerve length [40]-[42]. An

excursion occurs when the nerve has a gliding or a displacement relative to the surrounding nerve bed, his direction can be transverse or longitudinal, or a mixture of both, comparative to the nerve tract, the distance is measured in millimeters [43].

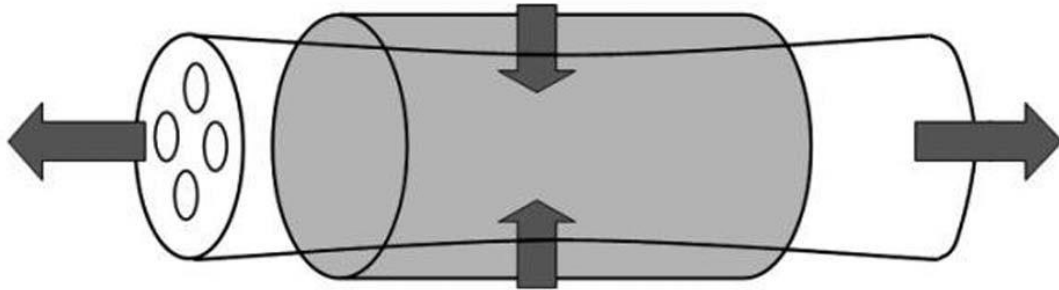


Figure 21 - Diverse types of stress applied on a peripheral nerve [43].

The mobility of peripheral nerves is one of the most important features. Consequently, this property allows the nerve to move together with or independently of adjacent structures, adapting their movements according to the primary tension and the stresses of specific anatomical locations, in certain cases they can slide on their internal process [24].

The nerve behaves as a semi-plastic material when imposed up to its elastic limit, at which the stress is proportional to strain. Thus, the nerve elongates fast without the further rise of the load until total rupture happens. The ulnar nerve trunk presents a maximal tensile strength of 1 to 2.2 kg/mm² and the median nerve 1 to 3.1 kg/mm² [38]. The ulnar nerve has a mean of 15% (range, 8%-21%) of elastic elongation limit; the mechanical failure happened at 18% (range, 9%-26%). The values for the median nerve are similar. They have a 6% ability to elongate without damage.

In the human body, the bony framework protects the nerves against elongation but in case of an unlimited traction force such as fracture, luxation or brachial plexus injury the nerves can break.

The normal sciatic nerve presents an elastic modulus of 40.96 ± 2.59 MPa and the Poisson's ratio (0.37 ± 0.02) obtained by strain electrical measurement. The nerve elastic modulus was determined according to Hooke's law:

$$E = \frac{\sigma}{\varepsilon} \quad (2.1)$$

In which E represents the elastic modulus, ε is the strain and σ refers to stress ($\sigma = P/A$; P : load; A : transverse section area). The Poisson's ratio was calculated based on the following equation [44]:

$$\mu = |\varepsilon^*/\varepsilon| \quad (2.2)$$

In which μ is the Poisson's ratio, ϵ^* corresponds to the transverse strain, and finally ϵ represents the longitudinal strain.

The peripheral nerve has two different options to adjust extension movements: either stretch or shift. The traction force which is longitudinal rises the intraneural pressure; due to the modification in the nerve cross-section. Thus, exists proportionally between the increase of the pressure and the reduction of the intersection. The nerves have an intrinsic capacity to make dynamic adjustment when occurs a nerve movement near its surroundings or by a modification inside the nerve itself [24].

2.5 - Constitutive Models

The peripheral nerve can be modulated as a homogenous and incompressible material so that it can be postulated as isotropic hyper-viscoelastic material. Thus, it is possible to study and describe the mechanical behavior of them. Using the most common polynomial hyperelastic strain energy function, used for tendons and nerves is possible to do that study [44], [45]:

$$\Psi(I_1) = c_1(I_1 - 3) + c_2(I_1 - 3)^2 + c_3(I_1 - 3)^3 \quad (2.3)$$

The $c_1, c_2, c_3 \in \mathbb{R}$ are scalar coefficients. The first strain invariant (I_1) and the deformation gradient $\mathbf{F}$ expresses the Cauchy stress tensor as follows [46]:

$$\sigma = -k\mathbf{I} + 2 \frac{\partial W(I_1)}{\partial I_1} \mathbf{F}\mathbf{F}^T \quad (2.4)$$

In which k is an indeterminate Lagrange multiplier, $\mathbf{I}$ is the unit tensor, and $\mathbf{F}^T$ corresponds to the transposed of the deformation gradient. Subsequently, $I_1 = tr(\mathbf{F}\mathbf{F}^T)$, and $\mathbf{F}$ were expressed as a function of principal stretches. The previous equation offers a theoretical connection between stress and stretch. The first strain invariant is written in function of the longitudinal stretch λ due to the assumption of the homogeneous triaxial stretch. The theoretical transverse stretch was $1/\sqrt{\lambda}$ regarding not only the incompressibility constraint but also the experimental boundary conditions. The longitudinal component of the Cauchy stress can be expressed as follows:

$$\sigma_z = \frac{\wp(\lambda, c_1, c_2, c_3)}{\lambda^3} \quad (2.5)$$

In which $\wp(\lambda, c_1, c_2, c_3) = 3c_3\lambda^9 + (2c_2 - 18c_3)\lambda^7 + 9c_3\lambda^6 + (27c_3 - 6c_2 + c_1)\lambda^5 + (2c_2 - 18c_3)\lambda^4 + (-27c_3 + 6c_2 - c_1)\lambda^2 + (36c_3 - 4c_2)\lambda - 12c_3$.

To conclude, the sensitiveness of the stress function with respect to c_1, c_2, c_3 can be expressed as: $SI(c_i) = (\sigma_{\max_{c_i}} - \sigma_{\min_{c_i}}) / \sigma_{\max_{c_i}} [47]$.

The $i = 1, 2, 3$, $\sigma_{\max_{c_i}}$ and $\sigma_{\min_{c_i}}$ correspond respectively to the maximum and the minimum values of stress for the maximum and minimum values of c_i , once the other constant had ideal values.

Chapter 3

Chitosan Biomaterial

In this chapter are presented not only the method of manufacture chitosan but also mechanical properties and some applications of the chitosan biomaterial.

The use of chitosan dates back to 1859 when a researcher discussed deacetylated forms of the parent chitin natural polymer [48]. Over the last 25 years, an extensive amount of studies of biomaterials and chitosan have been reported. The use of this biomaterial as a tissue support material occurs due to its physical, biological and chemical proprieties. Chitosan is a non-toxic hydrophilic polymer. They can be easily controlled and modified under certain conditions [16], [49]. Chitosan has exclusive biological properties such as biocompatibility, biodegradability, antibacterial, antitumoral, anticholesteremic, nontoxicity, high affinity to proteins, wound-healing agent, hemostatic and fungistatic properties [16]-[18], [50]. It is regularly used as a drug delivery carrier. Due to his natural source, chitin, the chitosan is cheap. It is derived from the exoskeleton of insects, crustaceans, and fungi. Chitin is the second primary natural organic source on the planet, immediately after cellulose.

Chitosan is a linear polysaccharide, a copolymer of N-acetyl-d-glucosamine and d-glucosamine units linked by β (1-4) glycosidic bonds, carbohydrate backbone is identical to cellulose. The content of glucosamine is responsible for the degree of deacetylation (DD). The molecular weight varies from 300 to over 1000 kD with a DD from 30% to 95% depending on the source and the preparation method. The chitosan has two hydroxyl groups and one amino group in the repeating glucosidic residue [50]. The degree of deacetylation influences several structural parameters and properties such as solubility, crystallinity, biodegradability, viscosity, biocompatibility, mucoadhesion, analgesic, antioxidant, hemostatic and antimicrobial.

When chitosan is in his crystalline form and an aqueous solution with pH seven is mostly insoluble, but when diluted with acids (pH 6) the protonated free amino groups on glucosamine facilitate solubility of the molecule. The strong inter and intramolecular hydrogen bonding between the chitin and chitosan chains influences and decreases their solubility in several organic solvents. The chitosan is usually provided with three different reactive functional groups: the amino group at C(2) and the primary and secondary hydroxyl groups at the C(3), and C(6) positions, respectively. The existence of the mentioned groups allows modifications

to native structure of chitosan which can be useful for tissue engineering applications such as scaffolds [16]. In Figure 22 is represented the chitosan structure [50].

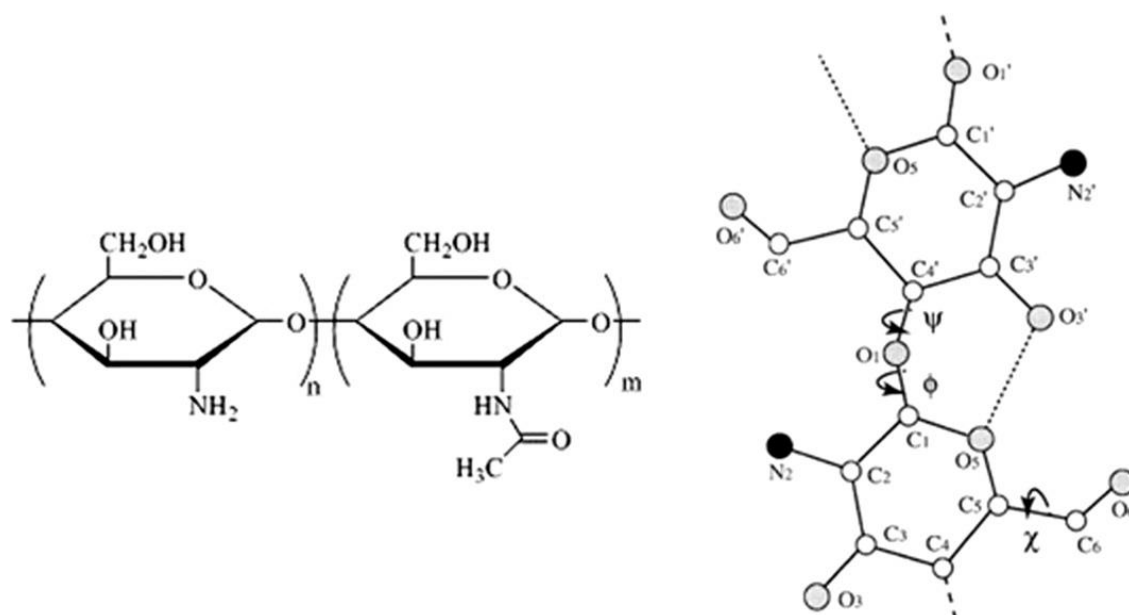


Figure 22 - Chemical structure of Chitosan [50].

The biodegradation plays an important role in the metabolic fate of chitosan. The human body can degrade the chitosan by two distinct ways: chemical or enzymatic biodegradation. The chemical degradation is associated with acid catalyzed degradation that occurs in the stomach. The other kind of degradation is enzymatic degradation, which consists of the hydrolyze of glucosamine-glucosamine, N-acetyl-glucosamine-N-acetyl-glucosamine and glucosamine-N-acetyl-glucosamine linkages predominantly by lysozymes and some bacterial enzymes in the colon. Both biodegradation processes would deliver fragments suitable for renal clearance. The degradation rate is a very important factor which influences the cellular process, including host response, cell growth and tissue regeneration. The DD influences the degradation rate inversely, a high degree of DD represents a low degradation rate of chitosan [51].

As chitosan displays a higher rigidity than nervous tissues, this polymer must suffer mechanical alterations to be used in nerve repair. Polymer combinations are the most effective method to originate new suitable polymers with better mechanical properties for tissue engineering applications. The chemical nature of this polymer offers several possibilities for ionic and covalent changes which allow a wide adjustment of mechanical and biological properties for tissue engineering.

3.1 - Chitosan properties and manufacturing process

There are several properties in chitosan-based scaffolds important in tissue repair. This polymer can be formed as interconnected-porous structures by two distinct ways: in the first

way chitosan needs to be freeze and lyophilize; in the second way, the chitosan suffers “internal bubbling process (IBP)”, where is formed chitosan- CaCO_3 gels with precise shapes using appropriate molds. This process needs the addition of CaCO_3 to the chitosan solution [16], [52]. There are several advantages in forming the chitosan as an interconnected-porous structure. In this structure, the cells can migrate and seed inside that. Thus, the cells number can grow up. The control of porosity and pore morphology in the chitosan scaffold influences not only in the control of cellular colonization organization and rate but also on the angiogenic process. These feature on chitosan polymer plays a major role in the improvement and development of appropriate scaffolds for tissue engineering [53]. This polymer can be used in various shapes, e.g., sponge, film or fiber[54].

Another essential property of chitosan consists of her cationic nature. Due to this property, the polymer allows pH-dependent electrostatic interactions with negatively charged species such as proteoglycans and glycosaminoglycans (GAG) spread along the body. The GAG controls the number of grow factors and cytokines such as heparan sulfate and heparin which represents an important advantage in tissue repairing because may retain and concentrate desirable factors secreted by colonizing cells [55].

The repair of nerve injuries has the main goal of directing the regenerating nerve fibers into the proper endoneurial tubes. The actual strategies for nerve repair as already mentioned in chapter 2, can be divided into two categories: (a) bridging, using grafting and tubulisation methods and (b) end-to-end suturing of the nerve ends. An extensive list of biomaterials has been recommended to produce artificial tubes for nerve repair, but there are several requisites because artificial tubes lack enough internal surface area for the adhesion of nerve fibers and Schwann cells (SCs). The effort of most researches in recent years consists of the study of bio-engineered nerve, particularly biodegradable [11]-[14], [56]

In vivo, the implanted biodegradable tubes may fail due to several factors such as the absence of internal support, the thin walls of the tube, the surrounding scar tissue constriction, muscle contraction, and body weight. To overcome the failure causes in nerve repair the artificial tubes must contain a biodegradable matrix to deliver an ideal structural, molecular and cellular framework for SCs and neurite migration across the nerve gap [16].

There are several strategies to produce chitosan tubes with polymer combinations. The preparation of Hydroxyapatite coated chitosan tubes for nerve repair including laminin-1 or laminin peptides as scaffolds is an example of a strategy which the chitosan tubes extracted from crab tendons with the elimination of proteins and calcium phosphate. The chitosan tubes to optimize the mechanical properties were submitted to heat press to transform the polymer in a triangular shape, after that the surface also has been modified with hydroxyapatite. When in vivo, all these preparations in the chitosan tube avoid the excessive swelling during the implantation process [57].

Due to the tube absorption of Laminin-1, YIGSR (Tyr-Ile-Gly-Ser-Arg) and IKVAV (Ile-Lys-Val-Ala-Val) a significant result was obtained, improving the expansion of regenerating axons in bridging of a 15 mm defect in the sciatic nerve. Through covalent bonding between the chitosan and laminin peptide, both molecularly aligned, the laminin peptide is immobilized. Thus, it was developed a new biomaterial for nerve repair [58]. The chitosan tubes give flexible to the peptides and allow a more effective interaction between the peptides and the cellular receptors, more than when the peptides where alone. The neurosteroids such as progesterone and pregnenolone, which are produced by SCs and delivered from chitosan scaffolds, offers a better nerve regenerative response accelerating the axonal regeneration [59].

Some researchers studied the differences between three different chitosan cross-linked films prepared with crosslinking agents. The first film of chitosan was crosslinked with hexamethylene diisocyanate (HDI), the second with epichlorohydrin (ECH) and the last one with glutaraldehyde (GA). Crosslinking decreases not only the degradation rate but also the swelling degree of the chitosan scaffold, However, the crosslinking increases the elastic modulus and the hydrophilicity of the polymer. Only the HDI cross-linked chitosan scaffolds improve the proliferation of SCs. The other cross-linked films do not promote the cell spread, and proliferation in some cases delay that.

The objective of some studies consists not only in blend chitosan with a peptide to turn similar the mechanical proprieties of scaffolds when compared with nerve tissues but also to improve nerve cell attachment. Chitosan/ poly(L-lysine) composite materials show a higher rate of attachment, differentiation, and growth of the cells in contrast to the cells cultured in simple chitosan membranes. This high rate occurs because of an increase in hydrophilicity due to the elevated concentration of hydroxyl groups and the positive surface charge of chitosan polymer. The composite film of chitosan with three wt.% poly(L-lysine) presents better results that collagen concerning cell affinity [60]. This polymer nowadays is already extensively used in tissue engineering. In Figure 23 is represented the attachment of PC12 cells (a cell line derived from a pheochromocytoma of the rat located in adrenal medulla) to the six different types of material [60].

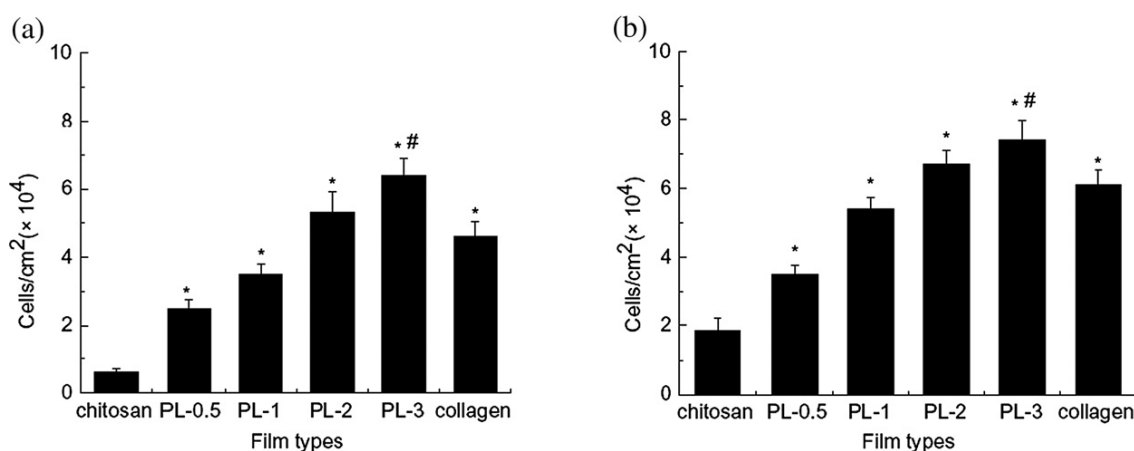


Figure 23 - Adhesion of PC12 cells to the different types of materials: (a) in serum free medium, (b) in medium containing 5% serum [60].

The addition of gelatin to chitosan polymer results in an improvement of nerve cell affinity due to several mechanical enhancements such as more elasticity and a high soft complex level. Thus, the mixture of chitosan with the gelatin composite causes a higher percentage of elongation at break and a minor modulus which promotes the PC12 cells to differentiate and expand more quickly than other chitosan films.

Chitosan supports nerve cell adhesion and neurite outgrowth which allows the use of them in scaffolds for neural tissue engineering.

3.2 - Mechanical properties of Chitosan

Several factors influence the mechanical properties of chitosan-based scaffolds such as the pore sizes and the orientation of the pores. The porous membranes of hydrated samples present a reduction of elastic moduli (0.1 - 0.5 MPa) when submitted to tensile testing. However, the non-porous chitosan membranes present a higher elastic modulus (5-7MPa) [61]. The porosity also influences the extensibility (maximum strain) of chitosan-based scaffolds, varying from different values depending on if the chitosan does not have porous (around 30%) or if they have, In the last case the values can reach higher than 100% as a meaning of both pore orientation and size. The maximum extensibility of chitosan was established with a structure frozen quickly at -78°C to give pores with 120 µm in diameter, the structure has random pore orientation. The stress-strain curve of porous chitosan corresponds to a typical composite materials curve, which can be divided into two different linear regions (Figure 24). The first region has a low modulus at low strains and the second region has a transition to an approximately two times higher modulus at the high strains [61].

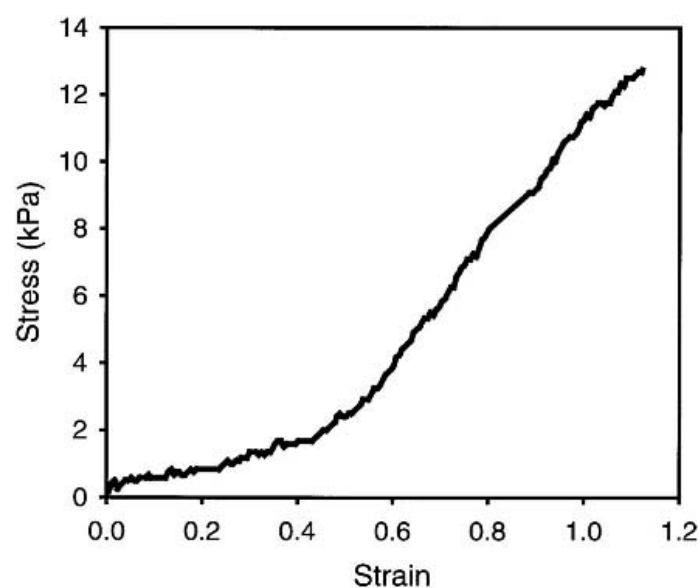


Figure 24 - Tensile stress-strain curve for a porous chitosan film [61].

The porous chitosan structures present a tensile strength that varies between 30-60 kPa [53], [61]. Another aspect that influences the mechanical proprieties of chitosan is her molecular weight and the crystallinity [62]. Chitosan with low molecular weight presents a lower tensile strength due to entanglement alterations (less entanglement). The low molecular weight also affects the crystallinity of the chitosan due to a low enthalpy of the subsequent film. Thus, a lower crystallinity causes a minor tensile strength of the film [61].

The Young's Modulus of pure chitosan is about 7.06 MPa [63]. The pH, in the fabrication process of chitosan, influences her mechanical proprieties, Young's Modulus is higher at pH7 when compared with pH9. When the chitosan film is prepared with hydroxyapatite particles, the Young's Modulus of the chitosan composites is always lower than pure chitosan. However, the addition of hydroxyapatite particles increases the breaking strength and strain of the chitosan composites [63].

Another important structural characteristic of chitosan that affects his mechanical proprieties is the degree of acetylation (DA). A decrease in DA will cause an increase in Young's modulus, tensile strength, and breaking elongation of the chitosan film [64].

The chitosan mechanical behavior when is fully swollen can be considered as analogous to rubber-like materials, which have a Poisson ratio of about 0.5 [65].

The next table summarizes the tensile proprieties of chitosan films [66]:

Table 3 - Summary of tensile properties of chitosan films [66].

	0.5% Chitosan film	1.5% Chitosan film	3.0% Chitosan film
Thickness (mm)	0.012 ± 0.0005	0.02 ± 0.0031	0.038 ± 0.0005
Young's modulus of elasticity (MPa)	4240 ± 80	4447.5 ± 160	4290 ± 110
Strain to failure (mm/mm)	0.011 ± 0.001	0.020 ± 0.002	0.027 ± 0.002
Yield stress (MPa)	39.8 ± 0.3	56.5 ± 5.9	62.7 ± 1.0
Elongation (%)	1.74 ± 0.03	1.59 ± 0.02	3.11 ± 0.03

3.3 -Constitutive models

The mechanical behavior of chitosan is better understood using the theories of rubber elasticity and viscoelasticity.

The chitosan can be postulated as a viscoelastic material. This polymer is mostly characterized as isotropic and incompressible material, which exhibit rubber-like characteristics. Thus, through Mooney-Rivlin equation the behavior of chitosan film can be characterized [67]. Typically, this kind of materials is referred as Mooney materials.

The work that is stored in the body of isotropic material as strain energy, W , can be express by the following most common equation, a power series expression:

$$W = \sum_{i,j,k=0}^{\infty} c_{ijk} (I_1 - 3)^i + (I_2 - 3)^j (I_3 - 1)^k \quad (3.1)$$

In which, I_1 , I_2 and I_3 are the strain invariants given by:

$$I_1 = \alpha_1^2 + \alpha_2^2 + \alpha_3^2 \quad (3.2)$$

$$I_2 = \alpha_1^2 \alpha_2^2 + \alpha_2^2 \alpha_3^2 + \alpha_3^2 \alpha_1^2 \quad (3.3)$$

$$I_3 = \alpha_1^1 \alpha_2^2 \alpha_3^3 \quad (3.4)$$

The Mooney-Rivlin equation consists of an expression that describes a uniaxial extension of an incompressible material, takes into account two different terms in the previous series (equation 3.1). The primary term is the lowest member of the previous series, $i = 1$, $j = 0$, and $k = 0$, and the next term is $i=0$, $j=1$, and $k = 0$. It is possible to obtain Mooney-Rivlin equation in case of uniaxial extension and letting $c_1 = c_{100}$ and $c_2 = c_{010}$ [67]:

$$\tau = \left(\frac{\partial W}{\partial \lambda} \right) = +2 \left(C_1 + \frac{C_2}{\lambda} \right) \left(\lambda - \frac{1}{\lambda^2} \right) \quad (3.5)$$

The stress-strain behavior of the material at higher elongations can be described by the Mooney-Rivlin. The previous equation (equation 3.5) can be modified in the next equation to predict better the behavior of swollen hydrogels such as chitosan:

$$\tau_s = 2C_1 v_r^{\frac{1}{3}} \left(\lambda_s - \frac{1}{\lambda_s^2} \right) + 2C_2 v_r^{\frac{5}{3}} \left(\lambda_s - \frac{1}{\lambda_s^3} \right) \quad (3.6)$$

The focus of viscoelasticity theory consists in the study of relationships between elasticity, molecular motion and flow in the polymeric materials [68]. The imposed mechanical motion interferes with the magnitude of the viscoelastic response in the material.

When mechanical stress (or strain) is applied in the polymer segments the chains move, and this process leads to a time dependency on the stress or strain. Thus, when the initial condition is removed an internal response is engendered by this movement which leads to a time-dependent response, resulting in a time-dependent recovery. When occurs full recovery, the behavior is considerate viscoelastic, but when the recovery is incomplete the material behavior is considerate viscoelastic-viscoplastic [68].

Chapter 4

Numerical Methods

The use of mechanical simulation is present in several fields of engineering such as in mechanical, civil, aerospace and automotive engineering. Nowadays, mechanical simulation is also used in health fields, surging the biomechanical simulation. Thus, using virtual models of humans or animals the biomechanical simulation solves some ethical and monetary issues related to the use of real models. These methods allow to save not only money and time but also solve complex problems with numerical approaches [69].

In this chapter, firstly, it is presented a brief description of finite element method (FEM) and some of its advantages and disadvantages. The second part of this chapter is based on a short explanation of two distinct meshless methods: RPIM and NRRPIM. The last part of this chapter is focused on solids mechanics where stress, strain, and displacement relationships.

4.1 - FEM

The use of FEM is important and normally indispensable in some virtually engineering analysis of structures, fluids or solids. This technique is capable of giving solutions for complex mechanical problems. The method is based on the simplification of a problem domain by domain discretization. Thus, the complex problem is simplified to simple and smaller domains called elements, which consist of a finite number of well-defined components called finite elements [70].

The FEM was developed to solve aerospace problems during the '60s but only in 1972, this method was used for human health applications. The first health field application of FEM was in studies of bone behavior. Thus, it was possible to understand not only the process of bone remodeling but also designing solutions to reduce the risk of fractures [69], [71].

There are several steps required for FEM modeling. Initially, it is necessary to represent the geometry of the model digitally. Thus, it is necessary to construct a virtual model by using computer aid design software like SolidWorks® or per segmentation of magnetic resonance (MR)

or computed tomography (CT) images. Then, it is necessary to define the proprieties of the material in the analysis which can be considered isotropic, anisotropic, transversely isotropic and orthotropic. The material properties influence the stress and strain distribution of the model. After defining the material proprieties, it is also necessary to define the boundary conditions and the load cases. The materials used in most of the study cases are characterized by two different constants: the Poisson's coefficient and Young's modulus; due to the material be assumed as homogenous, linear and with an elastic behavior.

The next step is the discretization which consists in dividing the entire domain into simple elements by the creation of a mesh with their respective elements and nodes. In Figure 25 is represented a creation of a mesh using FEM.

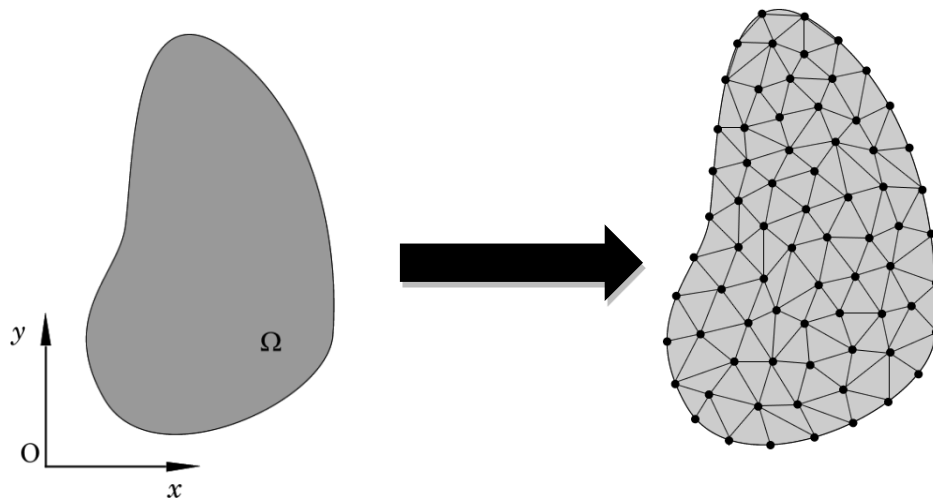


Figure 25 - Conception of a mesh using FEM.

The accuracy of the results depends on several parameters such as the type, total number, and arrangement of the elements (Figure 26). A high number of elements increases the accuracy but demands a bigger computational cost.

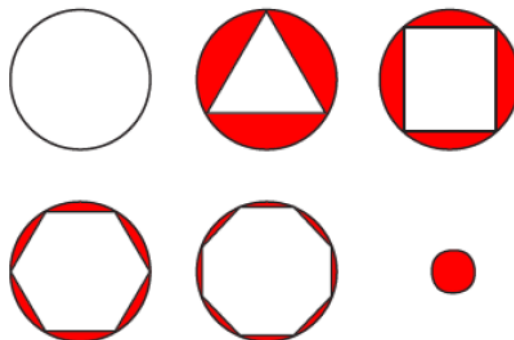


Figure 26 - Influence of the mesh on the discretization error [73].

It is possible the elements to have different proprieties and shapes, including irregular shapes allowing the discretization of structures composed of different materials [72].

The FEM can predict numerous variable fields when combined with the theory of elasticity, such as the strain, stress, and displacement. The assumptions made in the modeling of the structure such as the essential and natural boundary conditions, the materials properties and the geometry of the object in study influences the solution fields.

FEM has several advantages, they provide fast solutions with reasonable accuracy in an era where the speed is money, and the industry demands faster solutions, but this approach has certain limitations. In the health field, this method can be used to study a great range of biomedical engineering topics, such as bone remodeling, percutaneous heart valves; human lumbar spine; test scaffolds for tissue engineering; tissue modeling. With FEM it is possible to predict and interpolate the stress/strain state of any point inside the virtual geometric 3D or 2D model [25], [73], [74].

4.2 - Meshless Methods

The meshless methods in the last few years came into focus of the engineering community for solving a partial differential equation. Thus, these methods arise to overcome the gaps and difficulties of FEM mostly related to mesh generation [20], [75]

In Meshless methods when the domain of the problem is discretised the nodes are randomly distributed without any pre-established relation among them due to the field functions been approximated within an influence domain rather than an element. Thus, it is essential that influence-domains overlap each other. This typical procedure of these advanced methods are divided into three phases: the first one consists in the construction of the shape function, the second on the formulation and the last one is the integration procedure [20]. There are two different categories of meshless methods: approximation meshless methods and meshless interpolation methods.

The approximation methods were the first meshless methods; they use approximation functions to obtain smoother solutions. This advanced numerical method obtains the influence-domains through fixed radial searches. Using integration cells, this method constructs the background integration mesh, independent of nodal spreading, which is used to integrate the integral-differential equations that rule the study of a physical phenomenon. The meshless methods inherited the FEM integration pattern [20], [75]. The primary meshless approximation method was developed for astronomy, consists in the Smoothed-Particle Hydrodynamics (SPH), which was the foundation of the Reproducing Kernel Particle Method (RKPM) [76], [77].

Another important method, the first used for computational mechanics is the Diffuse Element Method (DEM) [78], which is based in the Moving Least Square approximants (MLS), proposed by Lancaster and Salkauskas for surface adjustment. Belytschko and his team developed one of the most popular and important meshless methods, the Element Free Galerkin Method (EFGM) by improving the DEM [79].

Although the approximation methods are effective and precise advanced discretization techniques, there are several computational problems such as in the imposition of essential and natural boundary conditions, due to the lack of the delta Kronecker property these

conditions had to be enforced by computational expensive numeric techniques (e.g., Lagrange multipliers) [20].

Interpolation methods have been developed to overcome the problems experienced by the approximation methods, surging several new methods in the last years such as the Point Interpolation Method (PIM), the Radial Point Interpolation Method (RPIM), the Natural Neighbour Finite Element Method (NNFEM), the Natural Neighbour Radial Point Interpolation Method (NNRPIM) and the Natural Element Method (NEM) [76]. The RPIM derivate from the PIM, by adding an extra functional base, RBF (Radial Basis Function). Natural Neighbour Radial Point Interpolation Method (NNRPIM) consists in a combination between NEM and RPIM [20].

The necessity of the dependency and construction of a background mesh for integration purposes causes much discussion in the researches community due to the relative false meshless discretization method. If the method requires a secondary mesh, background mesh, is not a truly meshless discretization method. The EFGM and the RPIM are examples of this kind of methods. Another option to avoid the false meshless discretization comes with NREM and NNRPIM where the integration mesh and the nodal connectivity only uses the nodal distribution. Thus, these methods are truly meshless methods [75], [80].

In this work are used only two different meshless methods: RPIM and RPIM, which will be explained in more detail in the next section.

4.2.1 - Common procedure in Meshless

The first approach to the problem consists in the study of the geometry, establish a solid domain and impose boundaries and boundary conditions. **Figure 27** represents these first steps [20].

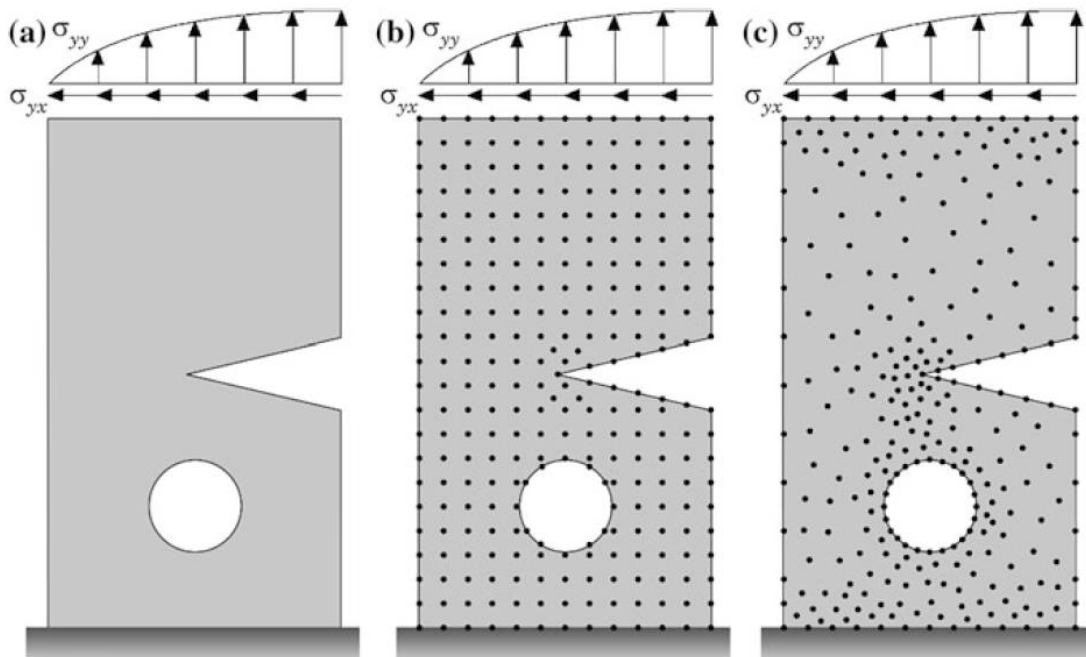


Figure 27 - Nodal discretization example: a) Solid domain. b) regular nodal discretization c) Irregular nodal discretization [20].

Afterward, the problem domain as shown in Figure 27(a) is numerically discretized through a nodal set. The nodal set can follow a regular (Figure 27 (b)) or irregular distribution (Figure 27 (c)). The unique information necessary by truly meshless methods is the spatial location of all nodes in the problem domain because this nodal distribution does not form a mesh. However, the nodal discretization has a direct effect on accuracy and performance of the method; a uniform nodal distribution leads to better and more accurate results [20].

The next step consists in the creation of nodal connectivity, using Voronoï diagrams or influence-domains. Thus, a background mesh can be created with the help of Gaussian integration meshes that have been adjusted and modified to the problem domain in the study.

Afterward, using interpolation or approximation functions, it is possible to find the field variables in study by the combination of RBFs with polynomial basis functions. In the interpolation method it is possible to use FEM techniques to impose boundary conditions due to delta Kronecker property. Thus, due to that propriety, the obtained function passes over all nodes inside the influence-domain.

Meshless methods have three different requirements: the first is the nodal connectivity, the second is the numerical integration scheme and the last are the shape functions. In the next sections, all these three requirements will be explained in more detail for RPIM and NNRPIM formulations since there are differences in nodal connectivity and numeric integration scheme. The sections about nodal connectivity, numerical integration and interpolation will be simplified using 2D domains. However, all the concepts introduced in the following sections are easily extended to higher dimensional spaces [20].

4.2.2 - Nodal Connectivity

4.2.2.1 - RPIM

The imposition of nodal connective between all the problem nodes is the second step after the discretization of the problem domain, but it is necessary in order to create that connectivity between nodes, the overlapping of the influence domains in each node.

From the exhaustive search of several nodes in determinate area or volume and with a fixed or variable shape and sizes comes up the influence domains. Figure 28 shows an example of two different types of fixed domains, in Figure 28 (a) is represented a rectangular and in Figure 28 (b) is represented a circular domain. Changing the size or the shape interferes with the nodal connectivity, resulting in a different one. Another parameter that influences the influence domains is the spatial distribution resulting in a different quantity of nodes. The final solution of the problem is influenced by all the factors mentioned before, and that complication might cause reduction of precision in the numerical method [20].

RPIM to overcome these problems relies on influence-domains with variable size. However, the influence domain has a constant number of nodes. Figure 28 (c) shows this process. This advanced numeric method uses the Galerkin weak formulation to find the discrete equation system. A polynomial basis and an RBF helps in the construction of the shape functions. Figure

28 (c) shows the radial search process using as the center a point of interest x_1 to find the n closest nodes. It is also showed the presence of constant nodal connectivity.

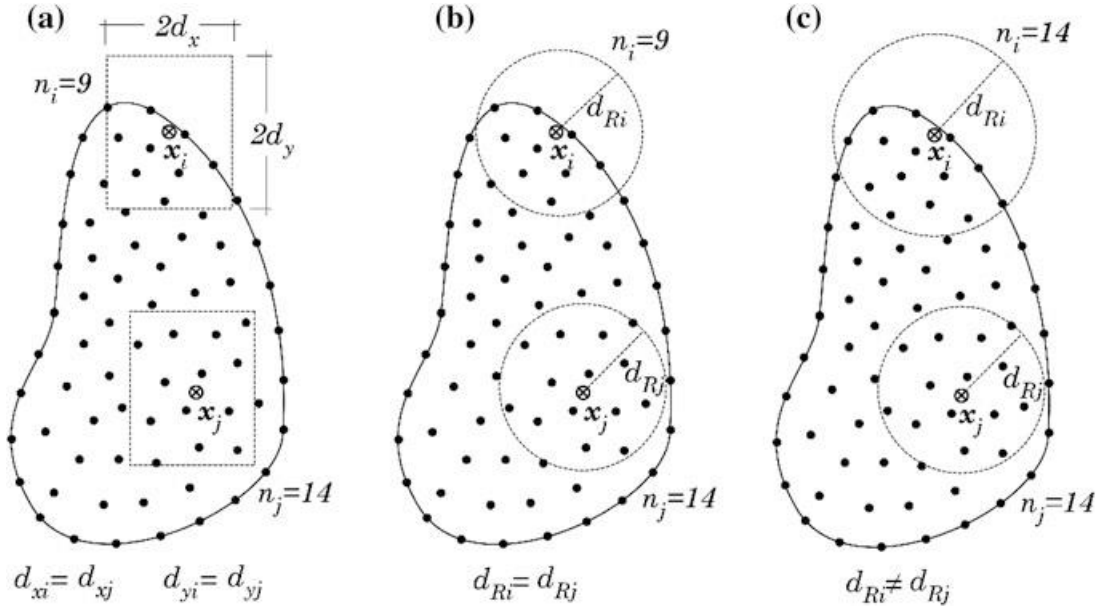


Figure 28 - Diverse types of influence domains: (a) Fixed rectangular shaped (b) Fixed circular shaped c Flexible circular shaped [20].

4.2.2.2 - NNRPIM

A new concept about nodal connectivity is implemented by NNRPIM, the natural neighbour. Thus, the natural neighbor is obtained using the Voronoï diagram which is composed of Voronoï cells where a single node is recognized in the discrete domain. Thus, in the NNRPIM the "influence-domain" is replaced by "influence-cells" [20].

The physical limit $\Gamma \in \Omega$ restricts the problem domain - $\Omega \subset \mathbb{R}^2$ - in NNRPIM. Thus, the domain is discretized in numerous randomly distributed nodes - $N = \{n_0, n_1, \dots, n_N\} \in \mathbb{R}^2$. The arbitrarily distributed nodes express the next coordinates: $X = \{x_0, x_1, \dots, x_N\}$, with $x_i \in \mathbb{R}^2$.

The Voronoï diagram of N establishes the partition of the problem domain. The Ω defines the problem domain which can be: convex and closed sub regions V_i . All these subregions V_i are connected through a node n_i . Therefore, each of these points inside V_i are closer to n_i than any other node, $n_j \in N \wedge i \neq j$. For $x_i \in \mathbb{R}^2$, The Voronoï cell, for $x_i \in \mathbb{R}^2$, is defined by the following equation [20]:

$$V_i := \{x_i \in \Omega \subset \mathbb{R}^2: \|x_i - x_i\| < \|x_j - x_j\|, \forall i \neq j\}, \quad (4.1)$$

The x_i refers to the interest point in study and $\|\cdot\|$ corresponds to the distance between the interest point and the nodes defined by the coordinates x_i and x_j [20].

The following equation is used to determinate the Voronoï diagram:

$$V = \{V_1, V_2, \dots\} \quad (4.2)$$

Figure 29 shows an analogous process how the Voronoï diagram [20]:

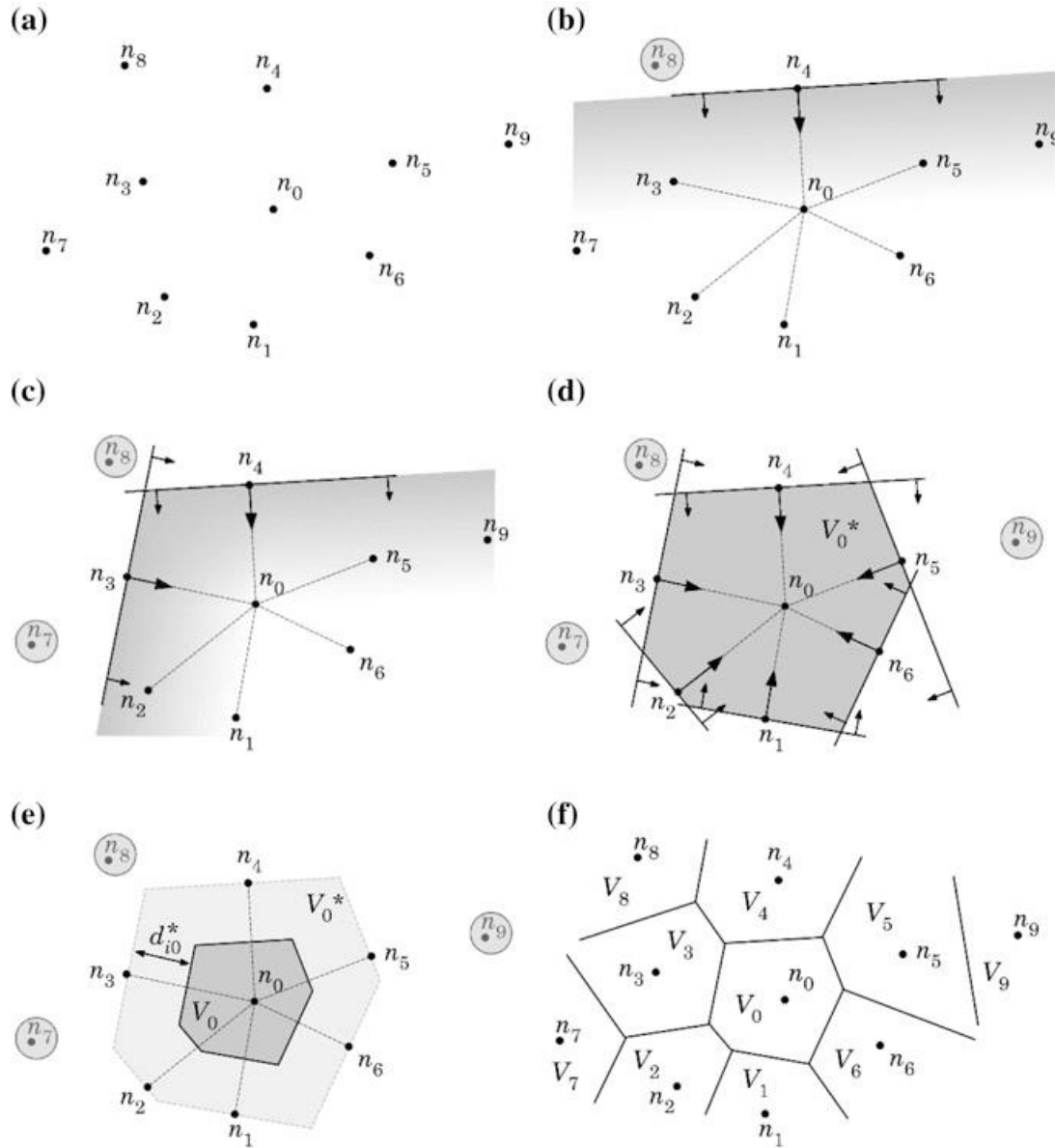


Figure 29 - (a) Initial nodal set of possible neighbour nodes (b) First trial plane (c) Second trial plane (d) Temporary cell containing just the natural neighbours (e) Node of Voronoï cell (f) Voronoï diagram [20].

The nodal set in Figure 29 is represented in 2D dimension, where is demonstrated the procedure of determining the Voronoï cell of node n_0 .

Primarily the neighboring nodes are chosen. Then, nodes that were not included in this early provisional selection are left out, and one of the selected nodes is designated as the potential neighbor. In Figure 29(b) the node n_4 is a case of this selection. Subsequently, after all the previous procedure the vector $\mathbf{u}_{40}$ can be defined by the following equation:

$$\mathbf{u}_{40} = \frac{(\mathbf{x}_0 - \mathbf{x}_4)}{\|\mathbf{x}_0 - \mathbf{x}_4\|} \quad (4.3)$$

Thus, $\mathbf{u}_{40} = \{x_{40}, v_{40}, w_{40}\}$ and a plane is established. There is an elimination of the nodes that do not follow the next conditions:

$$u_{40}x + v_{40}y + w_{40}z \geq (u_{40}x_4 + v_{40}y_4 + w_{40}z_4) \quad (4.4)$$

For all the nodes, that belongs to the primary set of nodes, the previous process is repeated in order to select the natural neighbors of node n_0 . This process is represented in Figure 29(c), Therefore, the provisional Voronoï cell is not only obtained but also converted into a final one as Figure 29(d) and Figure 29(e) illustrates. The following equation gives the distance among node n_0 and the limit of the Voronoï cell:

$$d_{oi}^* = \frac{d_{oi}}{2} = \frac{\|\mathbf{x}_0 - \mathbf{x}_1\|}{2} \quad (4.5)$$

The process of obtaining the remaining Voronoï cells is similar to the previous mentioned (Figure 29(f)) [20]. NNRPIM is based on the influence-cell concept, which implements a new concept of nodal connectivity. The overlap of the influence-cells, constructed based on the Voronoï diagram, carry out the nodal connectivity. Any point of interest can be interpolated by a certain cell with n nodes [80].

The influence cells can be divided into two different types: the first-degree influence-cells and the second-degree influence-cells which both are illustrated in **Figure 30**. The first type is established by the direct neighboring nodes when a point of interest x_I starts to search them (Figure 30 (a)). This process follows the Natural Neighbour Voronoï Construction.

The second-degree influence-cells depends on the first-degree as it showed in Figure 30 (b), a point of interest x_I searches neighbouring nodes like in the first-degree influence-cells. Subsequently, the natural neighbours of the first neighbours of x_I are attached to the influence-cell, this process considers the Voronoï diagram. The second-degree influence-cells allow to get more precise approximations due to his size be superior to the size of the first-degree influence-cells [20].

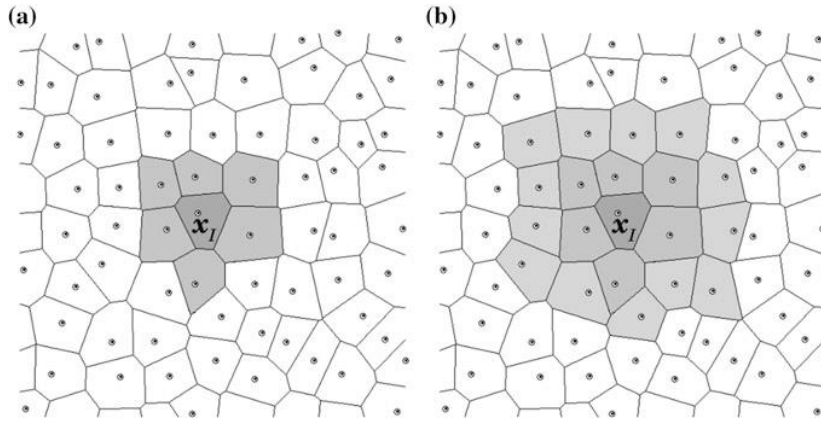


Figure 30 - (a) First-degree influence-cell; (b) Second-degree influence-cell [20].

4.2.3 - Numerical Integration

4.2.3.1 - RPIM

The RPIM is based on Gauss-Legendre quadrature procedure for numerical integration, following the next steps: Initially, using integration cells tailored to the solid domain (Figure 31(a)) or using a regular blind grid ((Figure 31 (b)) the background mesh is created. The second case, using a regular blind grid, the integration points outside the solid domain must be eliminated but in the first case, using an integration mesh fitted to the solid domain, in this process do not require any treatment. Thus, regarding the Gauss-Legendre quadrature rule, each cell is filled with integration points after setting the integration cells, these cells can be triangular or quadrilateral. [10]

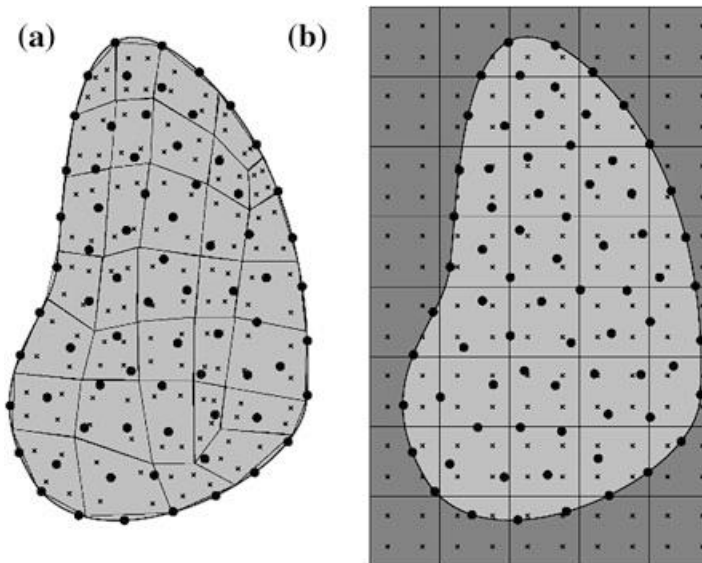


Figure 31 - (a) Fitted background mesh; (b) Regular grid integration mesh [20].

Figure 32 shows an example of a quadrilateral cell in the grid that was selected and then transformed into an isoparametric square. Thus, the integration points are distributed in that same square, in this case, a 2x2.

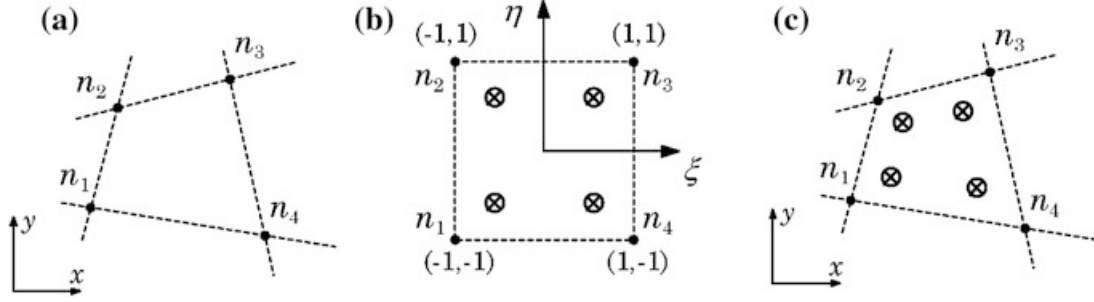


Figure 32 - (a) Early grid-cell; (b) Isoparametric square with integration points; (c) Initial cell with integration points [20].

Through isoparametric interpolation functions, N_i , for quadrilaterals is possible to obtain the Cartesian coordinates of the quadrature points:

$$\begin{aligned}
 N_1(\xi, \eta) &= \frac{1}{4}(1 - \xi)(1 - \eta) \\
 N_2(\xi, \eta) &= \frac{1}{4}(1 - \xi)(1 + \eta) \\
 N_3(\xi, \eta) &= \frac{1}{4}(1 + \xi)(1 + \eta) \\
 N_4(\xi, \eta) &= \frac{1}{4}(1 + \xi)(1 - \eta)
 \end{aligned} \tag{4.6}$$

In the case of triangles:

$$\begin{aligned}
 N_1(\xi, \eta) &= 1 - \xi - \eta \\
 N_2(\xi, \eta) &= \eta \\
 N_3(\xi, \eta) &= \xi
 \end{aligned} \tag{4.7}$$

The following equation gives the cartesian coordinates:

$$\begin{aligned}
 x &= \sum_{i=1}^m N_i(\xi, \eta) \cdot x_i \\
 y &= \sum_{i=1}^m N_i(\xi, \eta) \cdot y_i
 \end{aligned} \tag{4.8}$$

In the previous equation, the x_i and y_i represents the cartesian coordinates of the nodes which defines the integration cell. The m is the quantity of nodes inside the grid-cell. Multiplying the isoparametric weight of the quadrature point with the Jacobian matrix determinant of the own grid-cell allows to obtain the integration weight of the quadrature point:

$$J = \begin{bmatrix} \frac{\partial x}{\partial \xi} & \frac{\partial x}{\partial \eta} \\ \frac{\partial y}{\partial \xi} & \frac{\partial y}{\partial \eta} \end{bmatrix} \quad (4.9)$$

The following tables give the position and the weights of the isoparametric integration points for triangles and quadrilaterals:

Table 4 - Integration points and corresponding weights for triangles isoparametric cells.

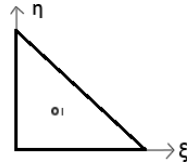
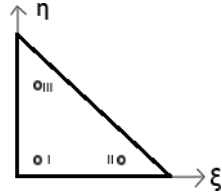
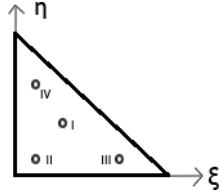
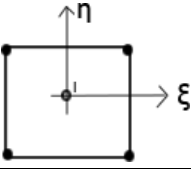
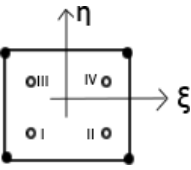
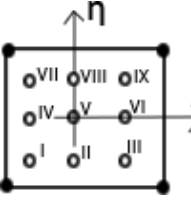
Gauss-Legendre Points	ξ	η	Weight	
I	$\frac{1}{3}$	$\frac{1}{3}$	$\frac{1}{2}$	
I	$\frac{1}{6}$	$\frac{1}{6}$	$\frac{1}{6}$	
II	$\frac{2}{3}$	$\frac{1}{6}$	$\frac{1}{6}$	
III	$\frac{1}{6}$	$\frac{2}{3}$	$\frac{1}{6}$	
I	$\frac{1}{3}$	$\frac{1}{3}$	$-\frac{27}{96}$	
II	$\frac{1}{5}$	$\frac{1}{5}$	$\frac{25}{96}$	
III	$\frac{3}{5}$	$\frac{1}{5}$	$\frac{25}{96}$	
IV	$\frac{1}{5}$	$\frac{1}{5}$	$\frac{25}{96}$	

Table 5 - Integration points and matching weights for quadrilaterals isoparametric cells.

Gauss- Legendre Point	ξ	η	Weight	
I	0	0	4	
I	$-\frac{1}{\sqrt{3}}$	$-\frac{1}{\sqrt{3}}$	1	
II	$+\frac{1}{\sqrt{3}}$	$-\frac{1}{\sqrt{3}}$	1	
III	$-\frac{1}{\sqrt{3}}$	$+\frac{1}{\sqrt{3}}$	1	
IV	$+\frac{1}{\sqrt{3}}$	$+\frac{1}{\sqrt{3}}$	1	
I	$-\sqrt{\frac{3}{5}}$	$-\sqrt{\frac{3}{5}}$	$\frac{25}{81}$	
II	0	$-\sqrt{\frac{3}{5}}$	$\frac{40}{81}$	
III	$+\sqrt{\frac{3}{5}}$	$-\sqrt{\frac{3}{5}}$	$\frac{25}{81}$	
IV	$-\sqrt{\frac{3}{5}}$	0	$\frac{40}{81}$	
V	0	0	$\frac{25}{81}$	
VI	$+\sqrt{\frac{3}{5}}$	0	$\frac{40}{81}$	
VII	$-\sqrt{\frac{3}{5}}$	$+\sqrt{\frac{3}{5}}$	$\frac{25}{81}$	
VIII	0	$+\sqrt{\frac{3}{5}}$	$\frac{40}{81}$	
IX	$+\sqrt{\frac{3}{5}}$	$+\sqrt{\frac{3}{5}}$	$\frac{25}{81}$	

4.2.3.1 - NNRPIM

The NNRPIM is also based in Galerkin weak form. Thus, this advanced numerical method also requires a background integration mesh. In this method is necessary to establish three fundamental parameters: nodal connectivity, integration points and shape functions, due to the lack of information beyond the spatial location of each node that discretizes the problem domain. Voronoï diagram construction allows getting an integration mesh that is defined taking into account the nodal distribution. Then using Delaunay triangulation, the area of each Voronoï cell is subdivided into several sub-areas. In Figure 33 is represented the process mentioned before [20].

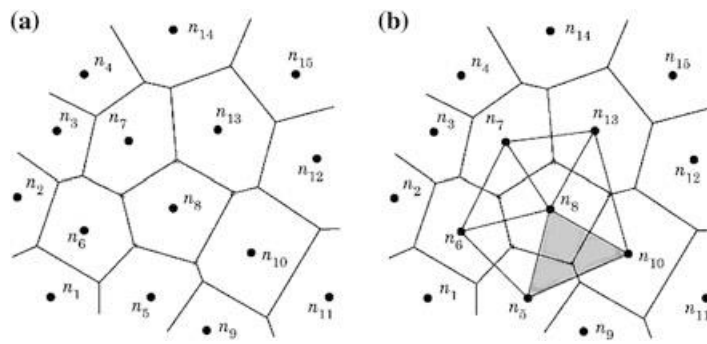


Figure 33 - Early Voronoï diagram. b Delaunay triangulation [20]..

Not only the corners of the polygon P_{Ii} are identified in the Voronoï cell but also the middle points, M_{Ii} , between n_I and the respectively neighbour node n_i are determined (Figure 34). Consequently, k quadrilateral sub-cells, S_{Ii} divides the Voronoï. The number of quadrilateral sub-cells created is equal to the number of natural neighbours of node n_I .

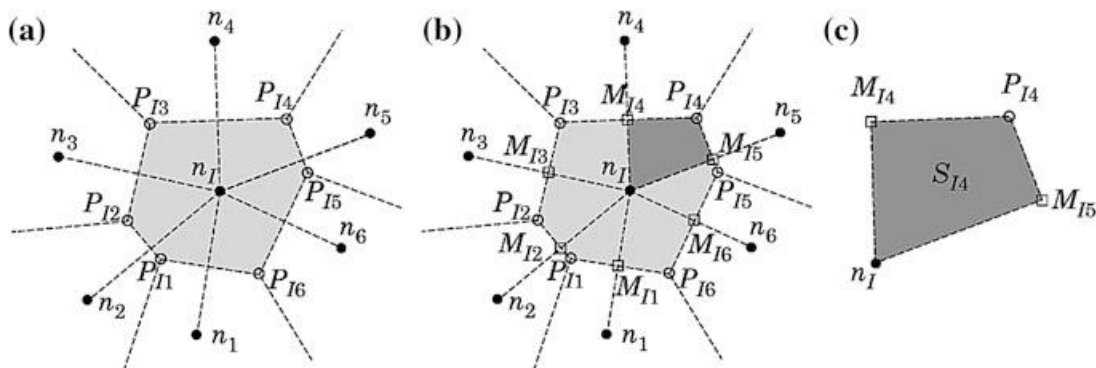


Figure 34 - (a) Voronoï cell and the corresponding intersection points, P_{Ii} ; (b) Middle points, M_{Ii} , and the respective generated quadrilaterals; (c) Quadrilateral [20]..

Figure 35 demonstrates the difference between nodes distributed in a regular and irregular way. In one hand on the irregular way, the spread N nodes forms quadrilateral sub-

cells. On the other hand, if the nodes are distributed in a regular way the sub-cells will have a triangular form.

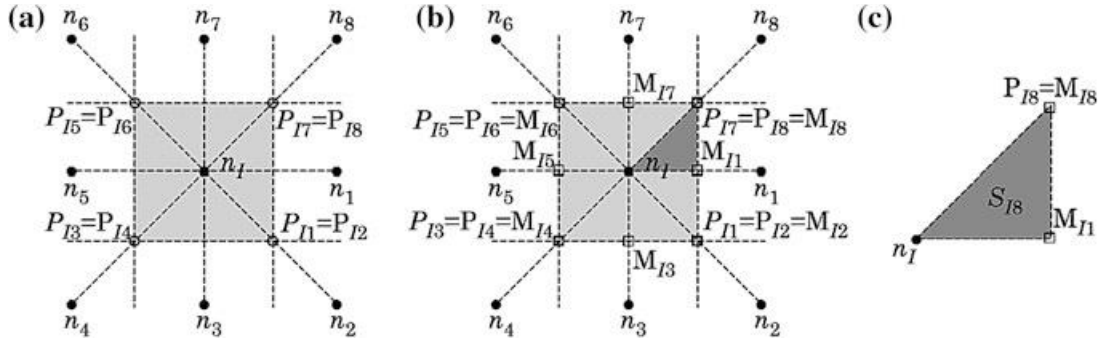


Figure 35 - Voronoi cell and the corresponding intersection points, P_{II} ; (b) Middle points, M_{II} , and the respective generated triangles; (c) Triangle [20].

Consequently, k sub-cells S_{II} , with a certain area $A_{S_{II}}$, divides the Voronoi cell area of interest point x_I . Thus, the sum of the area of each sub-cell is equal to the total area of the Voronoi cell, as shows the following equation:

$$A_{Vi} = \sum_{i=1}^n A_{S_{II}}, \forall A_{S_{II}} \geq 0 \quad (4.10)$$

Voronoi cell set consists in a portion of the domain, without gaps. Thus, the process can be applied to set sub-cells. Gauss-Legendre is used for numerical integration of the problem. There is a simple procedure in order to simplify the integration scheme, a point is placed in the centre of respectively quadrilateral or triangular sub-cells to obtain the spatial location of each integration point in each sub-cell as Figure 36. Illustrates [20], [76].

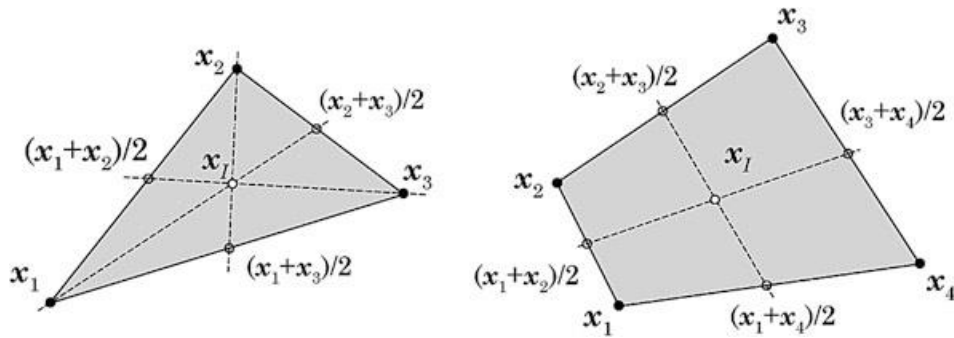


Figure 36 - Triangular and quadrilateral shapes and the corresponding integration points x_I [20].

However, by dividing the previously obtained sub-cells by crossing the center point with the middle points of the sub-cell cell edges, it is possible to obtain additional integration

points, which is represented in Figure 37. The integration process uses the Gauss-Legendre numerical integration to form the sub-quadrilaterals integration points. Figure 37 also represents the diverse integration schemes for the triangular and quadrilateral subcells, where the Voronoï cells, Δ , constitute the Voronoï diagram which defines the background integration mesh.

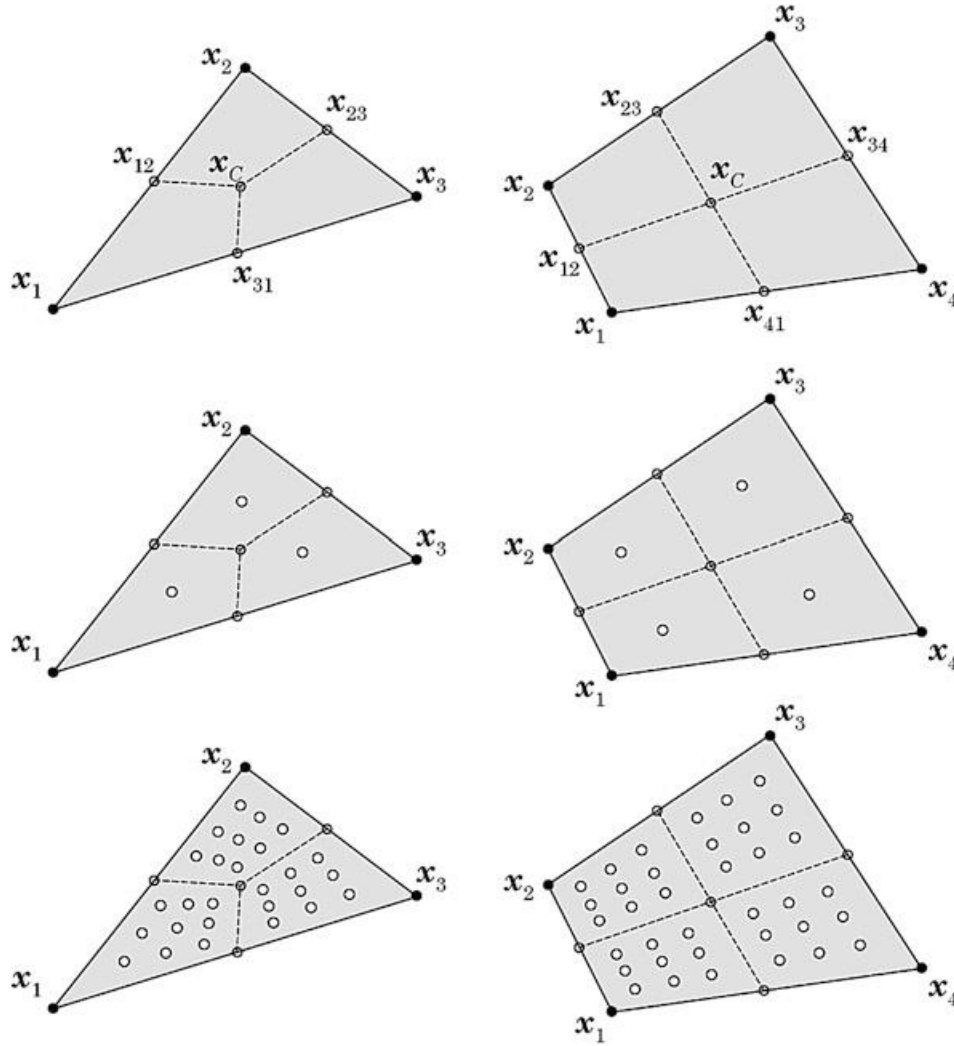


Figure 37 - Triangular and quadrilateral shapes - division, process in sub-cells and respective integration points x_i by the Gauss-Legendre integration scheme [20].

The present work uses only one integration point in the sub-area, $A_{S_{ij}}$, due to be sufficient to accurately integrate integral-differential equations according to several studies founded on literature [20], [76], [81].

4.2.4 - Interpolation Functions

RPIM and NNRPIM use RPI technique, by combining RBF and polynomial base functions, which is the equal process for interpolation functions in both methods. In the advanced

numerical methods previous referred, it is necessary to apply an interpolation method founded on the local nodal domain in order to overcome such problems as the absence of elements and to simplify the creation of the shape functions.

The delta Kronecker property is present in the interpolation functions of both methods: RPIM and NNRPIM, which consists of the following equation:

$$\varphi_i(\mathbf{x}_j) = \delta_{ij} = \begin{cases} 1 & (i = j) \\ 0 & (i \neq j) \end{cases} \quad i, j = 1, \dots, n \quad (4.11)$$

In the previous equation, δ_{ij} is the delta Kronecker. To numerically apply the essential boundary conditions in the stiffness matrix it is necessary the delta Kronecker property which allows direct imposition techniques when, $\delta_{ij}=1$ if $i=j$ and $\delta_{ij}=0$ if $i \neq j$.

The function $(\mathbf{x}_I)$ is defined in the domain Ω . This domain is discretized by several n nodes. However, $(\mathbf{x}_I)$ is influenced only by nodes within the influence-cell of the point of interest. The following expression gives the value of the function $(\mathbf{x}_I)$ when defined in the domain $\Omega \subset \mathbb{R}^2$, at the point of interest $\mathbf{x}_I$:

$$\mathbf{u}(\mathbf{x}_I) = \sum_{i=1}^n R_i(\mathbf{x}_I) \cdot a_i(\mathbf{x}_I) + \sum_{j=1}^m p_j(\mathbf{x}_I) \cdot b_j(\mathbf{x}_I) = \{\mathbf{R}(\mathbf{x}_I)^T, \mathbf{p}(\mathbf{x}_I)^T\} \begin{Bmatrix} \mathbf{a}(\mathbf{x}_I) \\ \mathbf{b}(\mathbf{x}_I) \end{Bmatrix}, \quad (4.12)$$

In the previous equation the $(\mathbf{x}_I)$ corresponds to RBF, $p_j(\mathbf{x}_I)$ is the polynomial function and $a_i(\mathbf{x}_I)$, n to the number of nodes inside the influence-cell of $\mathbf{x}_I$ and $b_j(\mathbf{x}_I)$ are the constant coefficients connected with the corresponding functions stated. [50] Generally, the $m < n$ to assurance a stable function.

The vectors mentioned in the previous equation (4. 12) can be represented as:

$$\begin{aligned} \mathbf{R}(\mathbf{x}_I) &= \{R_1(\mathbf{x}_I), R_2(\mathbf{x}_I), \dots, R_n(\mathbf{x}_I)\}^T \\ \mathbf{p}(\mathbf{x}_I) &= \{p_1(\mathbf{x}_I), p_2(\mathbf{x}_I), \dots, p_m(\mathbf{x}_I)\}^T \\ \mathbf{a}(\mathbf{x}_I) &= \{a_1(\mathbf{x}_I), a_2(\mathbf{x}_I), \dots, a_n(\mathbf{x}_I)\}^T \\ \mathbf{b}(\mathbf{x}_I) &= \{b_1(\mathbf{x}_I), b_2(\mathbf{x}_I), \dots, b_m(\mathbf{x}_I)\}^T \end{aligned} \quad (4.13)$$

The variable established in the RBF corresponds to the distance among the relevant node $\mathbf{x}_I$ and the neighbour node, which is given per $r_{II} = \|\mathbf{x}_I - \mathbf{x}_{II}\|$. The most common RBFs are the Multiquadrics (MQ) but several different RBFs were studied and developed. The common formulation of MQ-RBF is [82]:

$$R(r_{II}) = (r_{II}^2 + c^2)^p, \quad (4.14)$$

The value of c and p parameters varies between 0 and 1, respectively, to get precise results. The a and b are non-constant coefficients that require to be determined. The next monomial term defines the used polynomial basis functions [20]:

$$\mathbf{p}(\mathbf{x})^T = [1, x, y, x^2, xy, y^2, \dots] \quad (4.15)$$

To guarantee a unique solution is needed an extra equation, $\sum_{i=1}^n p_j(x_i) a_i(x_i) = 0$. Furthermore, is necessait ry to take in account the equation (4. 12) for each node inside the influence-cell. Thus, the follow system of equations is obtained:

$$\begin{bmatrix} \mathbf{u}_s \\ \mathbf{0} \end{bmatrix} = \begin{bmatrix} \mathbf{R} & \mathbf{P} \\ \mathbf{P}^T & \mathbf{0} \end{bmatrix} \begin{Bmatrix} \mathbf{a} \\ \mathbf{b} \end{Bmatrix} = \mathbf{M} \begin{Bmatrix} \mathbf{a} \\ \mathbf{b} \end{Bmatrix} \quad (4.16)$$

Where:

$$\mathbf{u}_s = \{u_1, u_2, \dots, u_n\}^T \quad (4.17)$$

$$\mathbf{R} = \begin{bmatrix} R(r_{11}) & R(r_{12}) & \dots & R(r_{1n}) \\ R(r_{21}) & R(r_{22}) & \dots & R(r_{2n}) \\ \vdots & \vdots & \dots & \vdots \\ R(r_{n1}) & R(r_{n2}) & \dots & R(r_{nn}) \end{bmatrix} \quad (4.18)$$

Thus, the constant polynomial basis can be defined as:

$$\mathbf{P} = [1 \quad 1 \quad \dots \quad 1]^T \quad (4.19)$$

The polynomial basis in the 2D problem is represented as:

$$\mathbf{P} = \begin{bmatrix} 1 & 1 & \dots & 1 \\ x_1 & x_2 & \dots & x_m \\ y_1 & y_2 & \dots & y_m \end{bmatrix}^T \quad (4.20)$$

However, in a 3D the polynomial function can be represented as:

$$\mathbf{P} = \begin{bmatrix} 1 & 1 & \dots & 1 \\ x_1 & x_2 & \dots & x_m \\ y_1 & y_2 & \dots & y_m \\ z_1 & z_2 & \dots & z_m \end{bmatrix}^T \quad (4.21)$$

The non-constant coefficients $\mathbf{a}$ and $\mathbf{b}$ can be determined by the following equation:

$$\begin{Bmatrix} \mathbf{a} \\ \mathbf{b} \end{Bmatrix} = \begin{bmatrix} \mathbf{R} & \mathbf{P} \\ \mathbf{P}^T & \mathbf{0} \end{bmatrix}^{-1} \begin{Bmatrix} \mathbf{u}_s \\ \mathbf{0} \end{Bmatrix} \Rightarrow \begin{Bmatrix} \mathbf{a} \\ \mathbf{b} \end{Bmatrix} = \mathbf{M}^{-1} \begin{Bmatrix} \mathbf{u}_s \\ \mathbf{0} \end{Bmatrix} \quad (4.22)$$

If change in equation (4. 12):

$$\mathbf{u}(x_I) = \{\mathbf{R}(x_I)^T, \mathbf{p}(x_I)^T\} \mathbf{M}^{-1} \begin{Bmatrix} \mathbf{u}_s \\ \mathbf{0} \end{Bmatrix} = \varphi(x_I) \begin{Bmatrix} \mathbf{u}_s \\ \mathbf{0} \end{Bmatrix} \quad (4.23)$$

Finally, for determinate the interpolation function in the interesting point, (x_I) :

$$\varphi(x_I) = \{\mathbf{R}(x_I)^T, \mathbf{p}(x_I)^T\} \mathbf{M}^{-1} = \{\varphi_1(x_I), \varphi_2(x_I), \dots, \varphi_n(x_I)\} \quad (4.24)$$

For further details, there are several studies in the literature that explains in more detail the construction and properties of the RPI and NNRPIM functions [20], [80], [83]

4.3 - Solid Mechanics

Solid Mechanics is the division of continuum mechanics that consists in the study of the behavior of solids under the action of loads, forces, temperatures changes, other external agents and phase changes, such as deformations and relative displacements of solids. Through this mechanical field is possible to know not only the connection between stress and strain but also between strain and displacements.

A material subject to different loads will have to distinct behaviors. It is important to know the basic concepts of stress and strain. Thus, in this subchapter is not only briefly explained the mentioned concepts but also the equilibrium and the constitutive equations. Solid Mechanics is essential for several fields of engineering such as aerospace, civil, nuclear, mechanical and biomedical.

4.3.1 - Basic Concepts

The stress-strain curve of each solid allows predicting his behavior, when is considered the solid characteristics and his structure configuration.

Although the real materials frequently exhibit non-linear behavior, in the present work the solids were considered linear-elastic due to ease of computation. Thus, for example, the relationship between stress and strain is supposed to be linear, so in this situation after elimination of the applied load, the solid returns to its primary form. In this work, only static loads were applied, so it is assumed a static nature. Another important feature is that this study assumes a static nature, which means that only static loads were considered and applied. Thus, the stresses, deformations, and displacements obtained do not depend on the time.

There are two different types of materials: isotropic and anisotropic. The anisotropic presents different properties in each direction. Thus, the deformation caused by the same load applied in different directions is not the same, so the proprieties vary methodically depending on the direction. The isotropic materials have uniformity in all directions. Thus, they only require two independent material properties: Young's Modulus (E) and the Poisson's ratio (ν).

When an analysis to study the solid deformation is performed, several factors must be taken in account: the stress and strain define the configuration change of the body, but it is necessary to ensure that the strain tensor and the stress tensor are in the same deformed state. The stresses of a present configuration can be characterized by the symmetric tensor of the Cauchy stresses:

$$\Lambda = \begin{bmatrix} \sigma_{xx} & \tau_{xy} & \tau_{xz} \\ \tau_{yx} & \sigma_{yy} & \tau_{yz} \\ \tau_{zx} & \tau_{zy} & \sigma_{zz} \end{bmatrix} \quad (4.25)$$

The tensors when are expressed in Voigt notation, they are expressed in column vectors which means a reducing in their order. Consequently, the stress tensor, (4.25), is altered into stress vector, σ :

$$\sigma = \{\sigma_{xx} \quad \sigma_{yy} \quad \sigma_{zz} \quad \tau_{xy} \quad \tau_{yz} \quad \tau_{zx}\}^T \quad (4.26)$$

There are two different components that stress can be divided: normal stress and shear stress. The first component, σ , is perpendicular to the plane in question. The shear stress, τ , is tangential to the plane in which it acts [84].

Thus, the strain vector $\boldsymbol{\varepsilon}$ appear by reduction of strain tensor $\boldsymbol{E}$:

$$\boldsymbol{\varepsilon} = \{\varepsilon_{xx} \quad \varepsilon_{yy} \quad \varepsilon_{zz} \quad \gamma_{xy} \quad \gamma_{yz} \quad \gamma_{zx}\}^T \quad (4.27)$$

The following constitutive equation is the Hooke's Law. This equation relates the stress with a strain of a solid domain in isotropic materials.

$$\boldsymbol{\sigma} = \boldsymbol{c}\boldsymbol{\varepsilon} \quad (4.28)$$

The $\boldsymbol{c}$ corresponds to the constitutive matrix which is calculated by $\boldsymbol{c}=\boldsymbol{s}^{-1}$, where the $\boldsymbol{s}$ consists in the compliance elasticity matrix. The $\boldsymbol{s}$ can be expressed by the following matrix for 3D cases of anisotropic materials in a plane stress formulation:

$$\boldsymbol{s} = \begin{bmatrix} \frac{1}{E_{xx}} & -\frac{\nu_{yx}}{E_{yy}} & -\frac{\nu_{zx}}{E_{zz}} & 0 & 0 & 0 \\ -\frac{\nu_{yx}}{E_{xx}} & \frac{1}{E_{yy}} & -\frac{\nu_{zy}}{E_{zz}} & 0 & 0 & 0 \\ -\frac{\nu_{xz}}{E_{xx}} & -\frac{\nu_{yz}}{E_{yy}} & \frac{1}{E_{zz}} & 0 & 0 & 0 \\ 0 & 0 & 0 & \frac{1}{G_{xy}} & 0 & 0 \\ 0 & 0 & 0 & 0 & \frac{1}{G_{yz}} & 0 \\ 0 & 0 & 0 & 0 & 0 & \frac{1}{G_{zx}} \end{bmatrix} \quad (4.29)$$

The E_{ii} corresponds to Young's modulus, G_{ij} is the distortion modulus of the material in direction i and j and ν_{ij} is the Poisson ratio.

For each point of the solid by considering the following displacements field:

$$\boldsymbol{u}(x, y, z) = \begin{pmatrix} u(x, y, z) \\ v(x, y, z) \\ w(x, y, z) \end{pmatrix} \quad (4.30)$$

The next expressions allow to calculate the components strain:

$$\begin{pmatrix} \varepsilon_{xx} \\ \varepsilon_{yy} \\ \varepsilon_{zz} \\ \gamma_{xy} \\ \gamma_{xz} \\ \gamma_{yz} \end{pmatrix} = \begin{pmatrix} \frac{\partial u}{\partial x} \\ \frac{\partial v}{\partial y} \\ \frac{\partial w}{\partial z} \\ \frac{\partial v}{\partial x} + \frac{\partial u}{\partial y} \\ \frac{\partial w}{\partial x} + \frac{\partial u}{\partial z} \\ \frac{\partial w}{\partial y} + \frac{\partial v}{\partial z} \end{pmatrix} \quad (4.31)$$

The strain is represented by two different types: the normal strain (ε), characterizes the relative change of length of a certain line segment and the shear strain (γ) represents the modification in the angle of two previously perpendicular line segments [85].

The combination of displacements field ($\mathbf{u}$) with a partial differential operator results in the strain vector:

$$\boldsymbol{\varepsilon} = \mathbf{L}\mathbf{u} \quad (4.32)$$

The $\mathbf{L}$ is obtained by:

$$\mathbf{L} = \begin{bmatrix} \frac{\partial}{\partial x} & 0 & 0 & \frac{\partial}{\partial y} & 0 & \frac{\partial}{\partial z} \\ 0 & \frac{\partial}{\partial y} & 0 & \frac{\partial}{\partial x} & \frac{\partial}{\partial z} & 0 \\ 0 & 0 & \frac{\partial}{\partial z} & 0 & \frac{\partial}{\partial y} & \frac{\partial}{\partial x} \end{bmatrix}^T \quad (4.33)$$

The equilibrium equations for a 3D example, considering an infinitesimal element, are obtained by the following equations:

$$\begin{aligned} \frac{\partial \sigma_{xx}}{\partial x} + \frac{\partial \tau_{xy}}{\partial y} + \frac{\partial \tau_{xz}}{\partial z} + F_x &= 0 \\ \frac{\partial \tau_{yx}}{\partial x} + \frac{\partial \sigma_{yy}}{\partial y} + \frac{\partial \tau_{yz}}{\partial z} + F_y &= 0 \\ \frac{\partial \tau_{zx}}{\partial x} + \frac{\partial \tau_{zy}}{\partial y} + \frac{\partial \sigma_{zz}}{\partial z} + F_z &= 0 \end{aligned} \quad (4.34)$$

4.3.2 - Strong form and Weak form

The strong form equations are ruled by a system of partial differential equations. Through these formulations, it is possible to find the exact solution, but this process has several limitations. It is a difficult task to solve this kind of equations, mainly due to complex engineering geometry problems. The weak form is established to overcoming the mentioned problems, it is constructed on a discrete system of equations and stated for each integration point. Weak form produces a higher algebraic systems stability, but it offers a minor consistency [20].

4.3.2.1 - Galerkin Weak form

The Galerkin weak form, which is a variational method created on energy minimization, allows obtaining the system of discrete equations.

A body in a domain Ω and bounded by Γ , where $\Gamma \in \Omega: \Gamma_u \cup \Gamma_t = \Gamma \wedge \Gamma_u \cap \Gamma_t = \emptyset$ is considered, where the Γ_u represents the essential boundary conditions and the Γ_t the natural boundary conditions. The external forces $\bar{t}$ and the natural boundary conditions are applied to the same surface of the body. Thus, there is a restriction of the displacements to the essential boundary conditions and where they were applied (Figure 38).

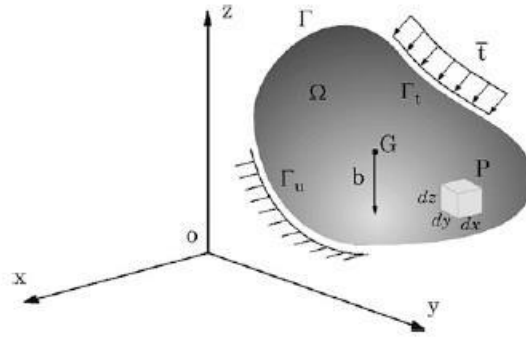


Figure 38 - Continuous solid body subject to volume and external forces [20].

The following equation creates the equilibrium equations that rule the linear elastostatic problem:

$$\nabla \Lambda + \mathbf{b} = 0 \quad (4.35)$$

As previously stated, the ∇ is the nabla operator, Λ is the Cauchy stress tensor and $\mathbf{b}$ is the body force per volume unit. The natural boundary follows a condition - $\nabla n = \bar{t}$ in Γ_t - which is different to the essential boundary condition - $u = \bar{u}$ in Γ_u , in which $\bar{u}$ corresponds to the displacement imposed at the essential boundary Γ_u .

The real solution minimizes the Lagrangian functional with the help of Galerkin weak form, recognized by:

$$L = T - U - W_f \quad (4.36)$$

In which the T refers to kinetic energy, U refers to strain energy and W_f is the work done by the external forces. The following expression defines the kinetic energy T :

$$T = \frac{1}{2} \int_{\Omega} \rho \dot{\mathbf{u}}^T \dot{\mathbf{u}} d\Omega \quad (4.37)$$

The Ω corresponds to the solid volume, the $\dot{\mathbf{u}}$ is the first derivative of displacement respecting the time and ρ corresponds to the solid mass density. In the case of the elastic materials the following equation gives the strain energy:

$$U = \frac{1}{2} \int_{\Omega} \boldsymbol{\varepsilon}^T \boldsymbol{\sigma} d\Omega \quad (4.38)$$

In the previous equation, the $\boldsymbol{\varepsilon}$ corresponds to the strain vector and the $\boldsymbol{\sigma}$ represents the stress vector. The following expression characterizes the work done by external forces:

$$W_f = \int_{\Omega} \mathbf{u}^T \mathbf{b} d\Omega + \int_{\Gamma_t} \mathbf{u}^T \bar{\mathbf{t}} d\Gamma_t \quad (4.39)$$

In which the $\mathbf{u}$ refers to displacement, $\mathbf{b}$ is the body forces and Γ_t corresponds to traction boundary where forces $\bar{\mathbf{t}}$ are applied. Consequently, the Galerkin weak form can be expressed by the following equation:

$$L = \frac{1}{2} \int_{\Omega} \rho \dot{\mathbf{u}}^T \dot{\mathbf{u}} d\Omega - \frac{1}{2} \int_{\Omega} \boldsymbol{\varepsilon}^T \boldsymbol{\sigma} d\Omega + \int_{\Omega} \mathbf{u}^T \mathbf{b} d\Omega + \int_{\Gamma_t} \mathbf{u}^T \bar{\mathbf{t}} d\Gamma_t \quad (4.40)$$

It is possible to minimize the previous Equation (4.40):

$$\delta \int_{t_1}^{t_2} \left[\frac{1}{2} \int_{\Omega} \rho \dot{\mathbf{u}}^T \dot{\mathbf{u}} d\Omega - \frac{1}{2} \int_{\Omega} \boldsymbol{\varepsilon}^T \boldsymbol{\sigma} d\Omega + \int_{\Omega} \mathbf{u}^T \mathbf{b} d\Omega + \int_{\Gamma_t} \mathbf{u}^T \bar{\mathbf{t}} d\Gamma_t \right] dt = 0 \quad (4.41)$$

After the change of variation operator δ inside the integrals:

$$\int_{t_1}^{t_2} \left[\frac{1}{2} \int_{\Omega} \delta(\rho \dot{\mathbf{u}}^T \dot{\mathbf{u}}) d\Omega - \frac{1}{2} \int_{\Omega} \delta(\boldsymbol{\varepsilon}^T \boldsymbol{\sigma}) d\Omega + \int_{\Omega} \delta \mathbf{u}^T \mathbf{b} d\Omega + \int_{\Gamma_t} \delta \mathbf{u}^T \bar{\mathbf{t}} d\Gamma_t \right] dt = 0 \quad (4.42)$$

The next equation represents the function integrated into the second integral:

$$\delta(\boldsymbol{\varepsilon}^T \boldsymbol{\sigma}) = \delta \boldsymbol{\varepsilon}^T \boldsymbol{\sigma} + \boldsymbol{\varepsilon}^T \delta \boldsymbol{\sigma} \quad (4.43)$$

In which $\boldsymbol{\varepsilon}^T \delta \boldsymbol{\sigma} = (\boldsymbol{\varepsilon}^T \delta \boldsymbol{\sigma})^T = \delta \boldsymbol{\sigma}^T \boldsymbol{\varepsilon}$. Through symmetry property of material matrix, $\mathbf{c}^T = \mathbf{c}$, and Hooke's law is possible to get:

$$\delta \boldsymbol{\varepsilon}^T \boldsymbol{\sigma} = \delta(\mathbf{c} \boldsymbol{\varepsilon})^T \boldsymbol{\varepsilon} = \delta \mathbf{c}^T \boldsymbol{\varepsilon}^T \boldsymbol{\varepsilon} = \delta \boldsymbol{\varepsilon}^T \mathbf{c} \boldsymbol{\varepsilon} = \delta \boldsymbol{\varepsilon}^T \boldsymbol{\sigma} \quad (4.44)$$

Consequently, the equation (4.44) is changed with:

$$\delta(\boldsymbol{\varepsilon}^T \boldsymbol{\sigma}) = 2 \delta \boldsymbol{\varepsilon}^T \boldsymbol{\sigma} \quad (4.45)$$

In this way, the equation (4.45) can be represented as:

$$-\rho \int_{\Omega} \delta(\mathbf{u}^T \dot{\mathbf{u}}) d\Omega - \int_{\Omega} \delta(\boldsymbol{\varepsilon}^T \boldsymbol{\sigma}) d\Omega + \int_{\Omega} \delta \mathbf{u}^T \mathbf{b} d\Omega + \int_{\Gamma_t} \delta \mathbf{u}^T \bar{\mathbf{t}} d\Gamma_t = 0 \quad (4.46)$$

Regarding not only the stress-strain relation, $\sigma = c\varepsilon$, but also the strain-displacement relation, $\varepsilon = Lu$, the previous equation (4.46) can be represented as:

$$\int_{\Omega} (\delta Lu)^T c(Lu) d\Omega - \int_{\Omega} \delta u^T b d\Omega - \int_{\Gamma_t} \delta u^T \bar{t} d\Gamma_t + \int_{\Omega} \delta(u^T \ddot{u}) d\Omega = 0 \quad (4.47)$$

Corresponding to a Galerkin weak form. This relation can be helpfully used for solid mechanical problems. For static problems, the fourth term of equation (4.47) is eliminated.

4.3.3 - Discrete equation system

The principle of virtual work serves as a base to obtain the discrete equation system for meshless methods. As previously mentioned, the interpolation functions are used to get field variables. The next equation shows how to obtain the field of displacements u , at an integration point x_I , for a 3D case:

$$u(x_I) = \sum_{i=1}^n \varphi_i(x_I) u(x_i) \quad (4.48)$$

The $\varphi_i(x_I)$ consists in the meshless approximation and, the (x_i) corresponds to the nodal displacement vector of n nodes contained in influence-domain of integration point x_I . Test (shape) functions allow to get Interpolated virtual displacement:

$$\delta u(x_I) = \sum_{i=1}^n \varphi_i(x_I) \delta u_i \quad (4.49)$$

In which $\delta u_i(x_i)$ represents the nodal virtual displacements. Merging the equation (4.47) with (4.49):

$$\begin{aligned} \int_{\Omega} L \left(\sum_{i=1}^n \varphi_i(x_I) \delta u_i \right)^T c L \left(\sum_{i=1}^n \varphi_i(x_I) u_i \right) d\Omega - \int_{\Omega} \left(\sum_{i=1}^n \varphi_i(x_I) \delta u_i \right)^T b d\Omega \\ - \int_{\Gamma_t} \left(\sum_{i=1}^n \varphi_i(x_I) \delta u_i \right)^T \bar{t} d\Gamma_t = 0 \end{aligned} \quad (4.50)$$

It is possible to rewrite the previous equation as a matrix equation. Thus, it is possible, for the point of interest x_I , to define the matrix of approximation/interpolation functions:

$$H(x_I) = \begin{bmatrix} \varphi_1(x_I) & 0 & 0 & \varphi_2(x_I) & 0 & 0 & \dots & \varphi_n(x_I) & 0 & 0 \\ 0 & \varphi_1(x_I) & 0 & 0 & \varphi_2(x_I) & 0 & \dots & 0 & \varphi_n(x_I) & 0 \\ 0 & 0 & \varphi_1(x_I) & 0 & 0 & \varphi_2(x_I) & \dots & 0 & 0 & \varphi_n(x_I) \end{bmatrix} \quad (4.51)$$

Thus, it is possible to rewrite the equation (4.50) as follows:

$$\delta \mathbf{u}^T \int_{\Omega} [(\mathbf{H}(\mathbf{x}_I))^T \mathbf{L}^T] \mathbf{c} [\mathbf{L} \mathbf{H}(\mathbf{x}_I)] d\Omega \mathbf{u} - \delta \mathbf{u}^T \int_{\Omega} (\mathbf{H}(\mathbf{x}_I))^T \mathbf{b} d\Omega - \delta \mathbf{u}^T \int_{\Gamma_t} (\mathbf{H}(\mathbf{x}_I))^T \bar{\mathbf{t}} d\Gamma_t = 0 \quad (4.52)$$

The influence-domain, taking into account $\mathbf{u}$ as the nodal displacement vector of entirely n nodes inside the influence-domain, can be written as follows:

$$\mathbf{u} = \{u_1, v_1, w_1, u_2, v_2, w_2, \dots, u_n, v_n, w_n\}^T \quad (4.53)$$

The multiplication between $\mathbf{L}$ and $(\mathbf{x}_I)$ defines the deformability matrix $\mathbf{B}(\mathbf{x}_I)$ for the interest point $\mathbf{x}_I$, where the partial differential operator matrix is the $\mathbf{L}$:

$$\mathbf{B}(\mathbf{x}_I) = \mathbf{L} \mathbf{H}(\mathbf{x}_I) = \begin{bmatrix} \frac{\partial \varphi_1(\mathbf{x}_I)}{\partial x} & 0 & 0 & \frac{\partial \varphi_1(\mathbf{x}_I)}{\partial y} & 0 & \frac{\partial \varphi_1(\mathbf{x}_I)}{\partial z} \\ 0 & \frac{\partial \varphi_1(\mathbf{x}_I)}{\partial y} & 0 & \frac{\partial \varphi_1(\mathbf{x}_I)}{\partial x} & \frac{\partial \varphi_1(\mathbf{x}_I)}{\partial z} & 0 \\ 0 & 0 & \frac{\partial \varphi_1(\mathbf{x}_I)}{\partial z} & 0 & \frac{\partial \varphi_1(\mathbf{x}_I)}{\partial y} & \frac{\partial \varphi_1(\mathbf{x}_I)}{\partial x} \end{bmatrix}^T \quad (4.54)$$

For $i = 1, 2, 3, \dots, n$, i.e., to the order of nodes inside of the influence-domain. Consequently, the matrix $(\mathbf{x}_I)$ is composed by the entire n matrices $\mathbf{B}_i(\mathbf{x}_I)$, one for each node that is inside of the influence-domain. To conclude, the equation (5. 28) is developed by substituting $\mathbf{L}(\mathbf{x}_I)$ by $\mathbf{B}(\mathbf{x}_I)$:

$$\delta \mathbf{u}^T \int_{\Omega} (\mathbf{B}(\mathbf{x}_I))^T \mathbf{c} \mathbf{B}(\mathbf{x}_I) d\Omega \mathbf{u} - \delta \mathbf{u}^T \int_{\Omega} (\mathbf{H}(\mathbf{x}_I))^T \mathbf{b} d\Omega - \delta \mathbf{u}^T \int_{\Gamma_t} (\mathbf{H}(\mathbf{x}_I))^T \bar{\mathbf{t}} d\Gamma_t = 0 \quad (4.55)$$

Consequently, it is obtained the following expression:

$$\int_{\Omega} (\mathbf{B}(\mathbf{x}_I))^T \mathbf{c} \mathbf{B}(\mathbf{x}_I) d\Omega \mathbf{u} - \int_{\Omega} (\mathbf{H}(\mathbf{x}_I))^T \mathbf{b} d\Omega - \int_{\Gamma_t} (\mathbf{H}(\mathbf{x}_I))^T \bar{\mathbf{t}} d\Gamma_t = 0 \quad (4.56)$$

Thus, the local static equilibrium equation is established for the influence-domain. Subsequently the first integral of equation (5. 32) corresponds to the local stiffness matrix, $\mathbf{K}$, and the local force vector $\mathbf{F}$ is divided in other two integrals correspond to $\mathbf{f}_b$ and $\mathbf{f}_t$, respectively.

The discrete global system of equations can be established by considering the nodal connectivity and the assembling of previous local matrices:

$$\mathbf{K} \cdot \mathbf{u} = \mathbf{F} \quad (4.57)$$

In which the $\mathbf{K}$ represents the local stiffness matrix, $\mathbf{u}$ the nodal displacement vector and finally $\mathbf{F}$ the global force vector.

Chapter 5

Numerical methods applied to nerve tissue

5.1 - FEM

Despite the fact that FEM was a relevant computational tool developed in the 1950s to perform calculations and solve aeronautic structural problems, nowadays it is used in health fields [25].

In the last years it can be verified that there was a massive increase of the published papers that relate the peripheral nervous system with the FEM, in the figure 39 it is represented a graph obtained in *Scopus* database through a research with ('FEM AND NERVE' or 'NUMERICAL ANALYSIS AND NERVE') keywords. Thus, this result indicates a considerable interest, with many unanswered questions and interesting topics to investigate.

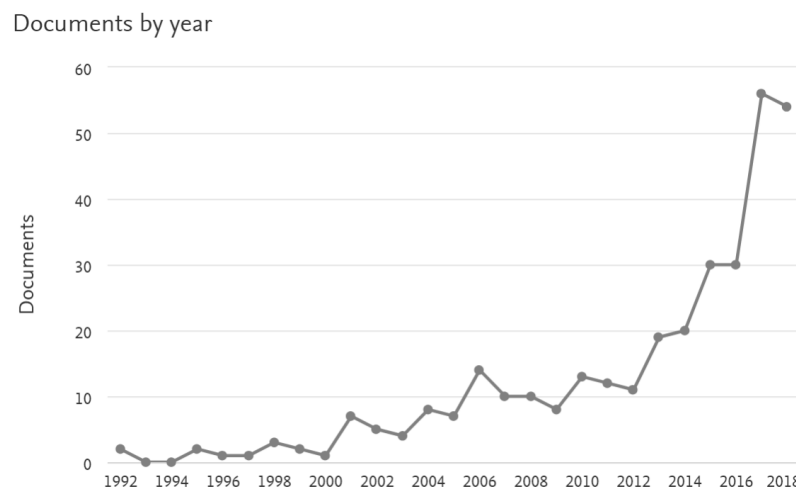


Figure 39 - Amount of papers published through the years founded in a research on Scopus database (www.scopus.com) assuming the keywords mentioned before.

Only in 1983 occurred the first study of compression effects on peripheral nerves using FEM, in which the researchers evaluated the effect of tourniquet application on the limb. The obtained results indicate that the calculated stresses were highest at the tourniquet edge. This study was consistent with photomicrographic and nerve conduction studies of damage caused by tourniquet use [86].

5.1 .1 - Geometrical Models

5.1 .1.1 - Two-dimensional

In the beginning, due to the little computation capacity of computers in, the first models were constructed in 2D to simplify the reality. However, these geometrical simplifications can lead to mathematical errors [87].

In a recent study, some researches investigate the mechanical properties of the ultrastructure of nerves by compressive response analysis and by simulation using hyper-viscoelastic finite element model (2D model). They conclude, using rat sciatic nerve that the shear stiffness of the nerve tissues decreases in the following order: perineurium, endoneurium, and epineurium. Thus, the first two structures give protection to the nerve fibers against compressive loads [88]. Figure 40 is represented the used 2D model in this study and the distribution of normal stress (σ_{yy}) after deformation, it is an example of a 2D model.

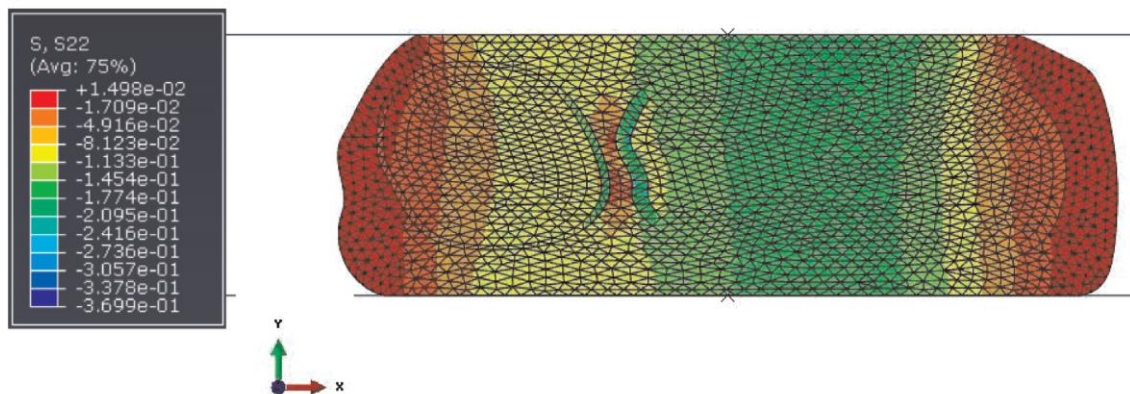


Figure 40 - Distribution of normal stress (σ_{yy}) in the deformed nerve ultrastructure [88].

Cheolwoong and Brown tried to estimate mechanical stress on the median nerve of the carpal tunnel using numerical formulation. In a first approach, the researchers used a 2D model where the carpal tunnel wall was defined as rigid to simplify. Thus, through FE stress results they may offer a basis for better identifying risk factors of individual subjects anatomy, and for studying biomechanical proprieties of clinical interventions [89].

Some studies are using FEM to characterize chitosan scaffold mechanical behavior, nonetheless, literature has flaws in this subject [90], [91]. The study of biomechanical behavior

of NGC's made with chitosan using FEM still haven't been done. Thus, it is important to study and understand this subject to improve the mechanical proprieties of the tubes.

5.1 .1.2 - Three-dimensional

Due to the increase in computational capacity, it is possible to analyze more complex models that are more similar to reality, 3D models.

The study of Intraneural Ganglion Cysts (IGC), which results in denervation of the muscles innervated by the cystic in common peroneal nerve had been done using FEM trough simulation process and using pressure and displacement boundary conditions. The following Figure 41 represents the used model used in section [92].

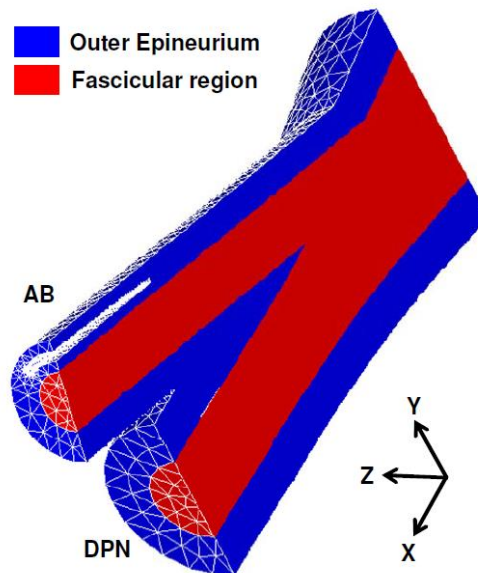


Figure 41 - Example of a 3D FE model [92].

Some researches investigate through a 3D finite element model the traumatic spinal cord injury (SCI) in rats. The model was taken from MR and CT images its consists in distinct element sets for the gray and white matter, the cerebrospinal fluid (CSF), the dura mater, a rigid rat spinal column, and a rigid impactor. The model allows predicting stress and strain patterns that corresponding patterns of the main injury, and also showed that separate descriptions of the material and/or failure properties of gray and white matter are important inclusions [93].

The study previously mentioned in section 5.1.1.1, where researches want to estimate the mechanical stress on the median nerve of the carpal tunnel using numerical formulation also use 3D models [89]. There are several recent studies of optic nerve because the excessive intraocular pressure (IOP), which has been considered the principal mechanism of glaucoma, and thus an initiator of blindness due to optic nerve damage that interrupts transmission of electrical impulses from the retina to the brain [94], [95].

5.1.2 - Boundary conditions

In most cases of FEM analyses, it is only considered a small part of the peripheral nerve in the study.

The imposition of the boundary conditions depends on the type of simulation in the study, a compression test may have different restrictions when compared to a tensile test. The simulations must take in account the real conditions of a chitosan tube in a human body.

5.1.3 - Constitutive models

The peripheral nerve can be postulated as isotropic hyper-viscoelastic material and modulated as a homogenous and incompressible material. Thus, it is possible to study the mechanical behavior of them using the most common polynomial hyperelastic strain energy function, used for nerves and tendons is possible to do that study [44], [45]

The chitosan can be assumed as a viscoelastic material and in most of the cases as isotropic and incompressible material. This kind of constitutive model is selected to best fit the multi-axial behavior of the chitosan film under mechanical stress. Thus, the Mooney-Rivlin equation can characterize the mechanical behavior of chitosan film [67]. In a recent study, four different isotropic models were analyzed for a biomechanical test of a chitosan film. The models are Mooney-Rivlin, neo-Hookean, Yeoh model, and Ogden model. The first and the last mentioned models have obtained the best results in representing the nonlinear behavior of the chitosan tube [91].

5.1.4 - Structural analysis

There are three major types of structural analysis: static, dynamic and contact. The type of analysis performed on the structure in the study depends on the nature of the loading. In the static analysis, loads vary slowly. Consequently, it can be assumed that static loads continue constant and with the time evolution, there is no substantial variation in the structural response. In the case of dynamic, the loads are transient loads vary significantly with time. Therefore, there is a need to calculate the structural response in every time instant. In a recent study the mechanical proprieties of a peripheral nerve ultrastructure were investigated based on an optical coherence tomography (OCT) image. The nerve was exposed on compression experiments in a dynamic test [88].

5.2 - Meshless

As mentioned in Chapter 1, after deep research in several scientific databases such as *ScienceDirect*, *Scopus*, *Elsevier*, and *Springer*, it is possible to verify that only appears a reference to a single paper, so, meshless methods have not much used for study the biomechanical behavior of chitosan NGCs for peripheral nerve regeneration. In the previously mentioned paper, some researches constructed several different geometric models of chitosan tubes for nerve repair, and then they analyzed numerically using different meshless methods and FEM. The obtained results show the structural response of these scaffolds when submitted to flexion, where the results obtained with FEM and with meshless methods are very similar which confirms the robustness of the used methods [3]. Despite the positive results obtained by that researches, these advanced numerical methods are appropriate for the study of structural simulation of chitosan tubes.

Most of the work founded in the literature about meshless was done to validate this approach, by comparing the results with other stable numeric methods such as FEM.

These advanced numerical techniques demonstrated to be valuable for several fields of biomechanics such as dental implants and bone remodeling [20], [96]. Thus, meshless methods allow and are capable of studying the different properties and behaviors of chitosan guide tube by doing different simulations and using a different design of the tube.

Therefore, due to the lack of documents regarding the study of chitosan guide tubes using meshless methods, this work proposes to fulfill that gap.

Chapter 6

Preliminary Research Work

This chapter consists of the preliminary research work performed to acquire technical competences and a proficient level in numerous computational mechanics software, such as:

- 3D slicer, which allows to segment DICOM files and to construct 3D discretize models from medical images.
- MeshMixer, which allows manipulating STL files and smooth shapes.
- SolidWorks®, to construct the 3D model.
- FEMAP, which allows to mesh surface domains and improve and remesh 3D domains.
- FEMAS, which allows performing several computational mechanics analyses (such as elastic analysis; elasto-plastic analysis) using both FEM and meshless methods.

6.1 - Construction of the model

Using a patient's CT scans (DICOM extension), it is possible to build the three-dimension (3D) model in 3DSlicer® of the peripheral nerve bifurcation, however, due to the difficulty in finding CT scans with excellent resolution (because the nerve is partially soft tissue), the 3D model was built in SolidWorks®. The model was created in SolidWorks® according to the real dimensions, then after the creation of the model it is saved as an STL file. To smooth the model and improve its aspect, the STL file was imported into Meshmixer®. After saving the model in a new STL file, it was imported to Femap® with NX Nastran to create a mesh and define the material and property of the model. A new two-dimensional (2D) model was created in Femap® with NX Nastran. Finally, it was exported the two INP files that were placed in FEMAS® (Finite Element and Meshless Analysis Software - cmech.webs.com). After that, the boundary conditions and forces were imposed (it will be described further). Figure 42 shows the different steps for the construction of the final model.

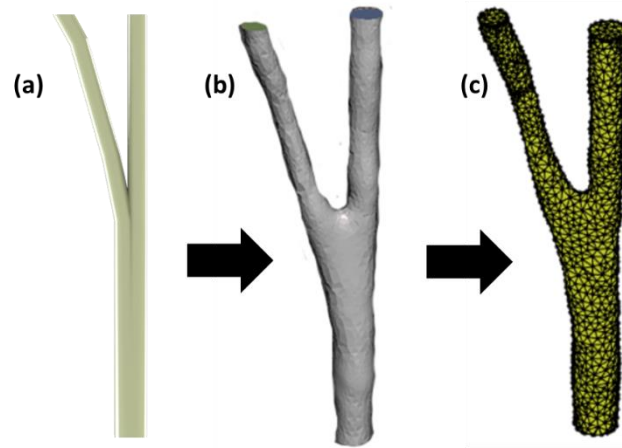


Figure 42 - Evolution of the model: (a) 3d model built in SolidWorks®; (b) After smooth the model in Meshmixer®; (c) Final model in FEMAS after the creation of the mesh in FEMAP®.

6.2 - Model Proprieties and Analysis

The 2D model in the study is composed by a mesh of 892 nodes and 1533 elements. As shown in Figure 43, this model has only one patch (Patch 1), the main model dimensions are also represented in Figure 43. The nerve bifurcation has 1.17mm high and 4mm wide, representing the main section of the bifurcation.

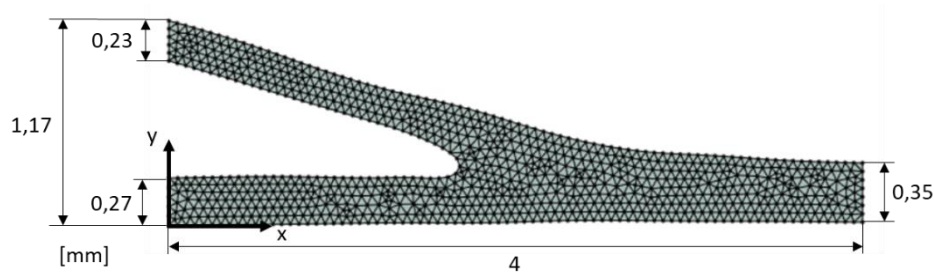


Figure 43 - Peripheral nerve bifurcation bidimensional model with main dimensions.

The 3D model is composed by a mesh of 2466 nodes and 9887 elements. This model has only one patch (Patch 1), the same as the 2D model, the main dimensions are the same represented in Figure 43. The model is represented in Figure 44 with the referential axis.

Both models were constructed for the analysis, considering the geometry of the real peripheral nerve bifurcation. The model was created using a preprocessing software (FEMAP®), as mentioned before. For this work, it was necessary to determine the physical and mechanical properties of the two models. The material was considered isotropic. Consequently the mechanical properties of a material point are the equivalent in all directions

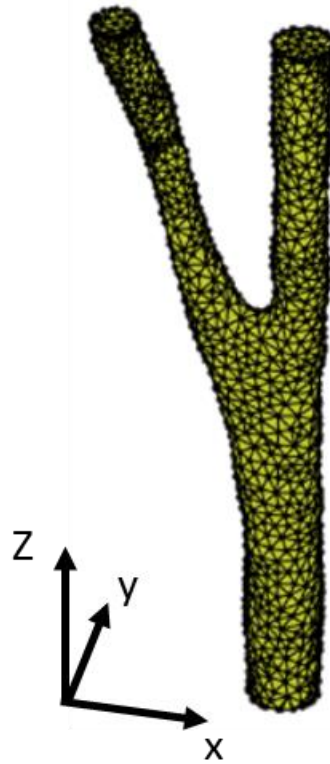


Figure 44 - Peripheral nerve bifurcation 3D model.

The properties which have been taken into account for the material were the Poisson's coefficient (ν) and Young's modulus (E), these mechanical properties are shown in **Table 6**.

Table 6 - Mechanical properties of the material.

Material	Young Modulus (Mpa)	Poisson's Ratio	Literature Ref.
Peripheral nerve tissue	40.96	0.37	[44]

This work considers the von Mises maximum stress criterion; this principle is founded on the Mises-Hencky theory, also identified as shear energy theory or maximum distortion theory. In this theory, a malleable isotropic material begins to yield in a location where the von Mises tension becomes equal to the limit of its tension [97].

For the case in study, the boundary conditions considered for 2D and 3D models were applied on the end of peripheral nerve bifurcation, in the both nerves, avoiding either the movement or the rotation in any direction, as shown in Figure 45 (a) for 2D model and in Figure 45 (b) for 3D model.

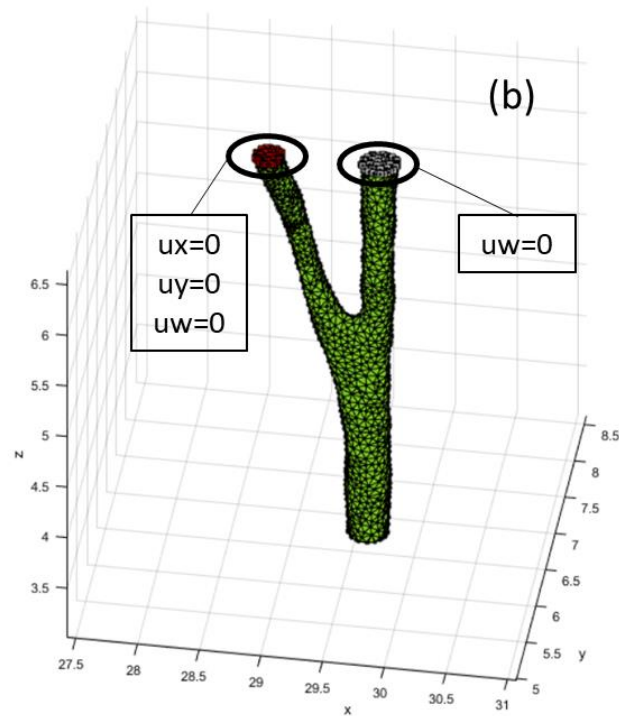
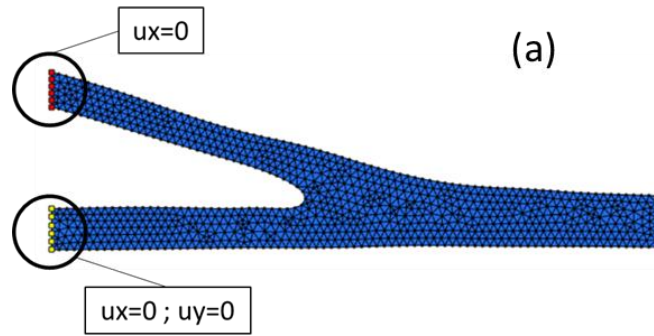


Figure 45 - Boundary conditions on: (a) 2D model and (b) 3D model.

For the stress analysis, there was considered one stretching case: a force of 0.423N was applied in the extremity of the peripheral nerve according to [98] (Figure 46).

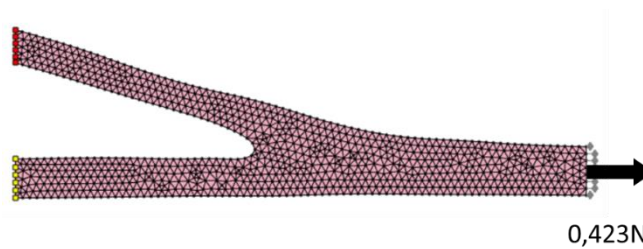


Figure 46 - Force applied in the 2D and 3D model.

To study the displacement of the bifurcation of the peripheral nerve, two random points were selected in the terminal zone where the stretch tension was applied. The selected points for the two-dimensional and three-dimensional model are represented in Figure 47.

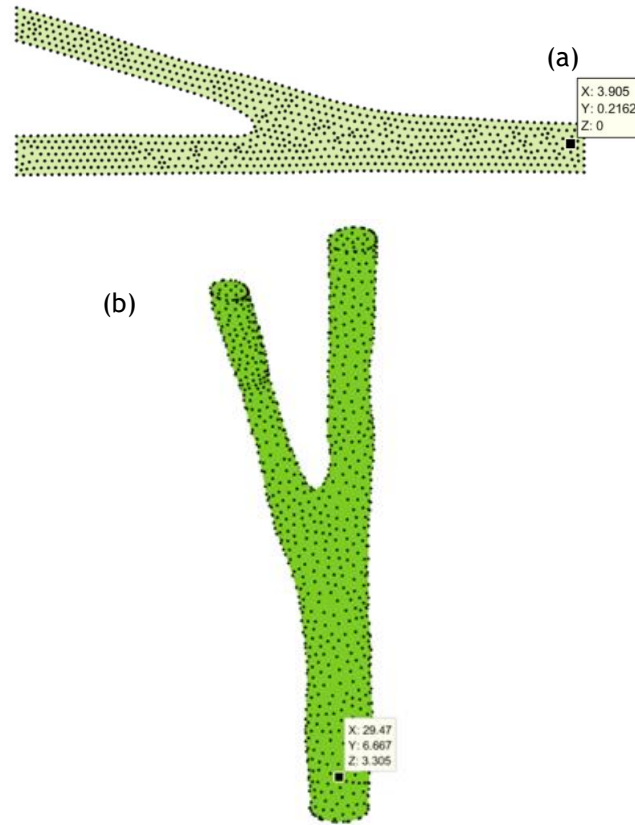


Figure 47 - Selected points to displacement analysis in the 2D (a) and 3D (b) model.

The elastostatic study was performed with the requirements previously presented using the three different methods, the results obtained for displacement analysis are shown in Table 7.

Table 7 - Total displacement (mm) for the selected points (PA and PB) in the two models ($|u|$).

	FEM	RPIM	NNRPIM
2D	1.085	1.154	1.061
3D	0.619	0.831	0.808

The results obtained for the von Mises effective von Mises stress (σ_{ef}) are presented, in stress color maps for both models according to the used method, in Table 8. The principal stresses (σ_{11} , σ_{22} , σ_{33}) results are showed in Table 9, Table 10 and Table 11 also in stress color maps.

Table 8 - Effective von Mises stress of both models.

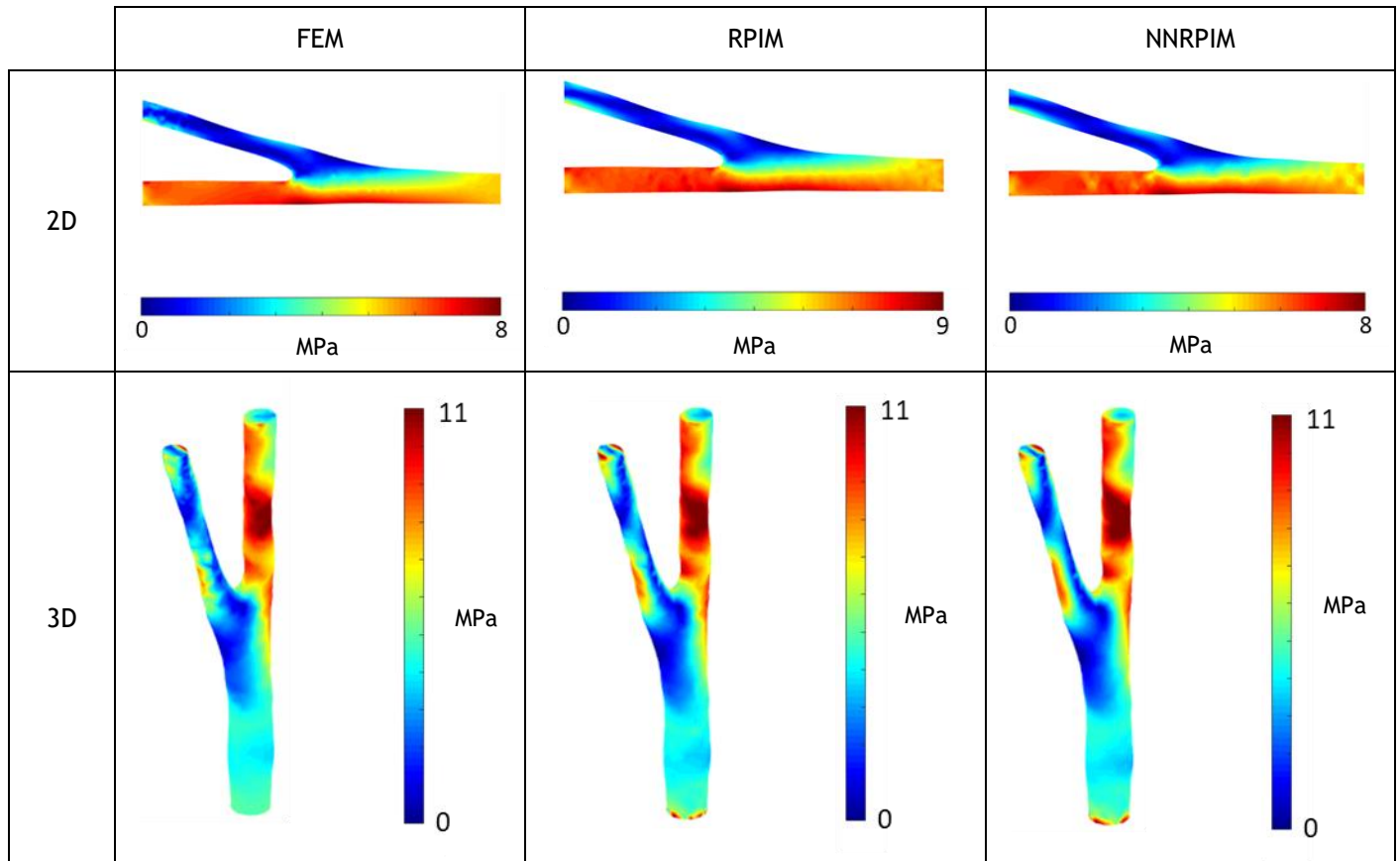


Table 9 - σ_{11} stress of both models.

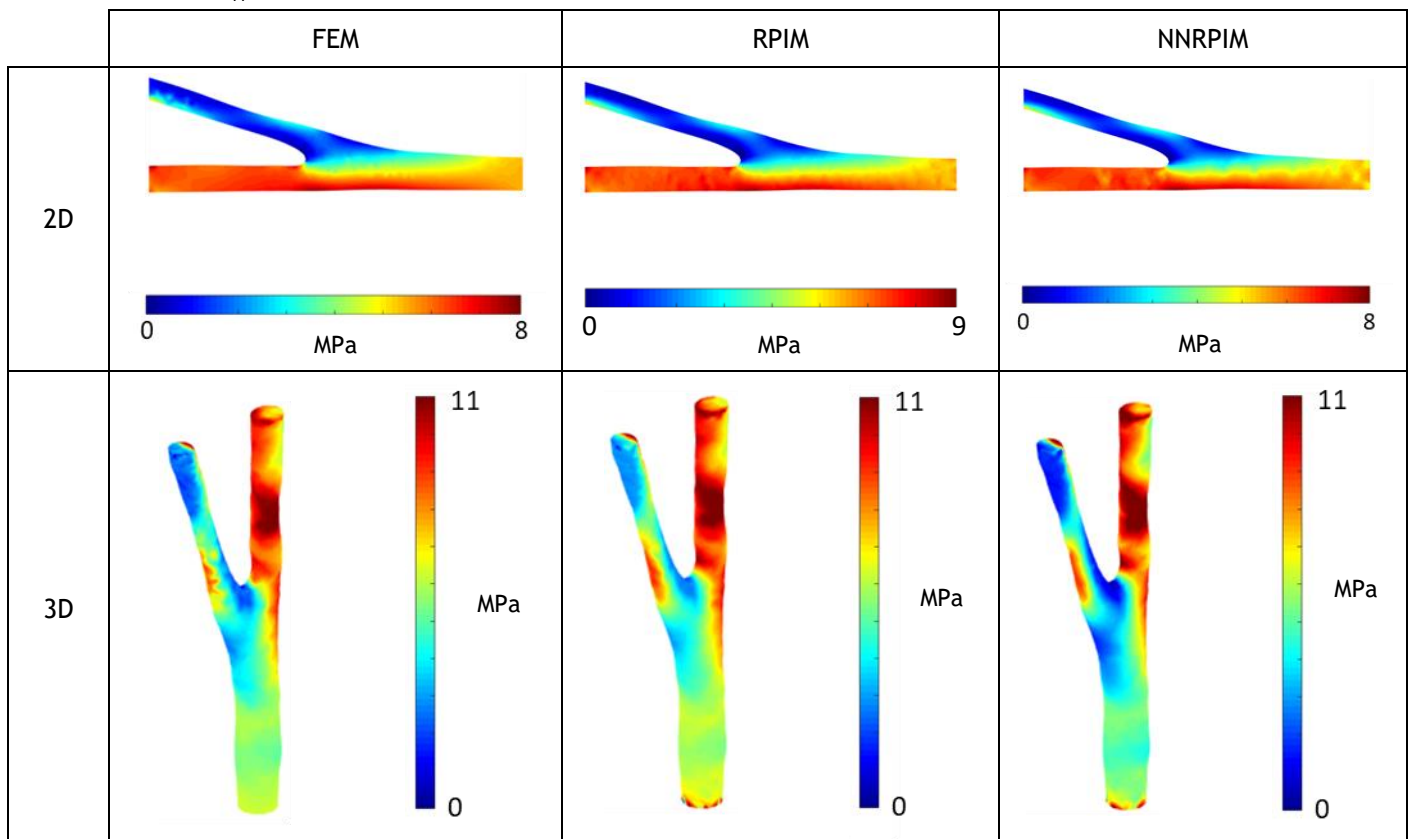


Table 10 - σ_{22} stress of both models.

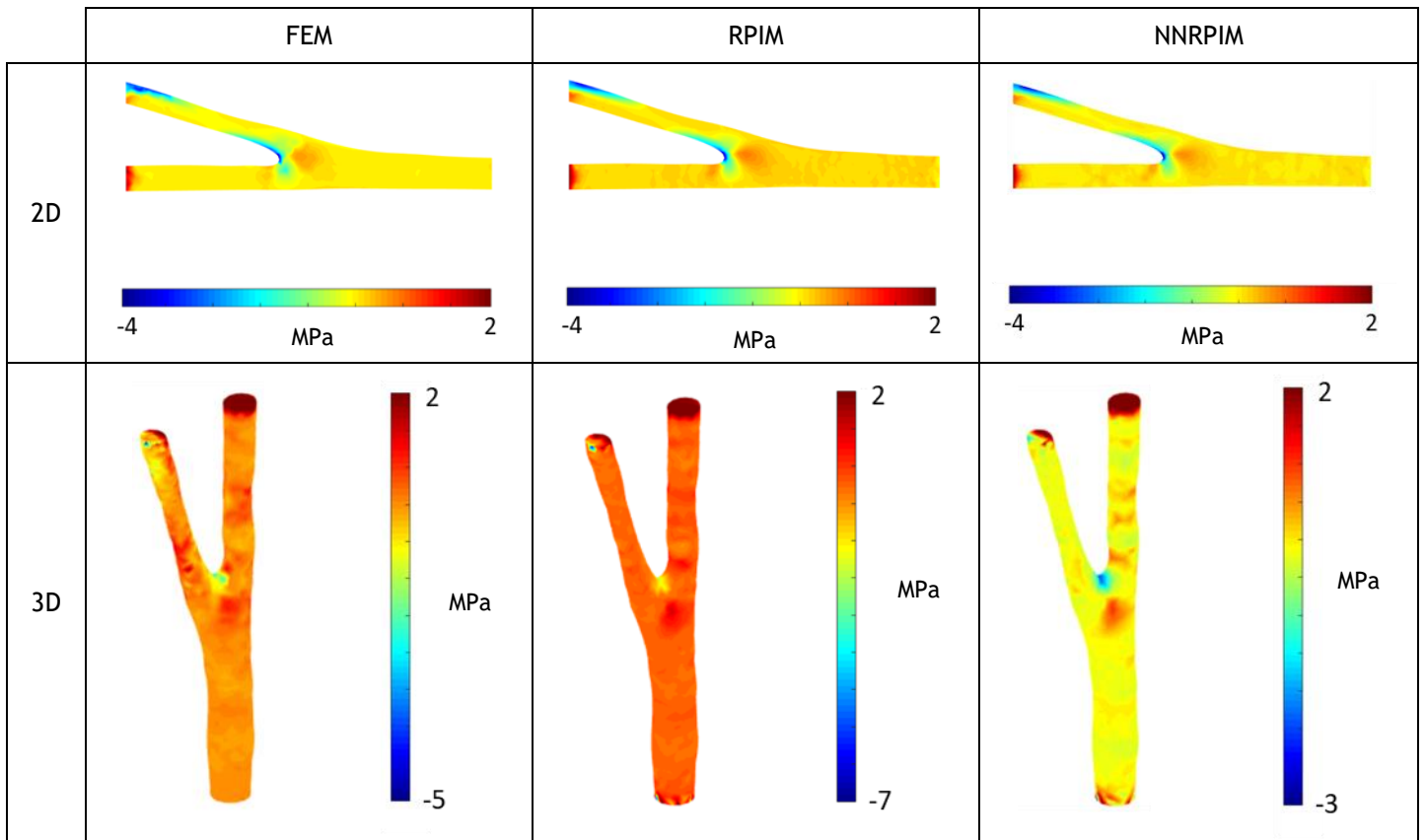
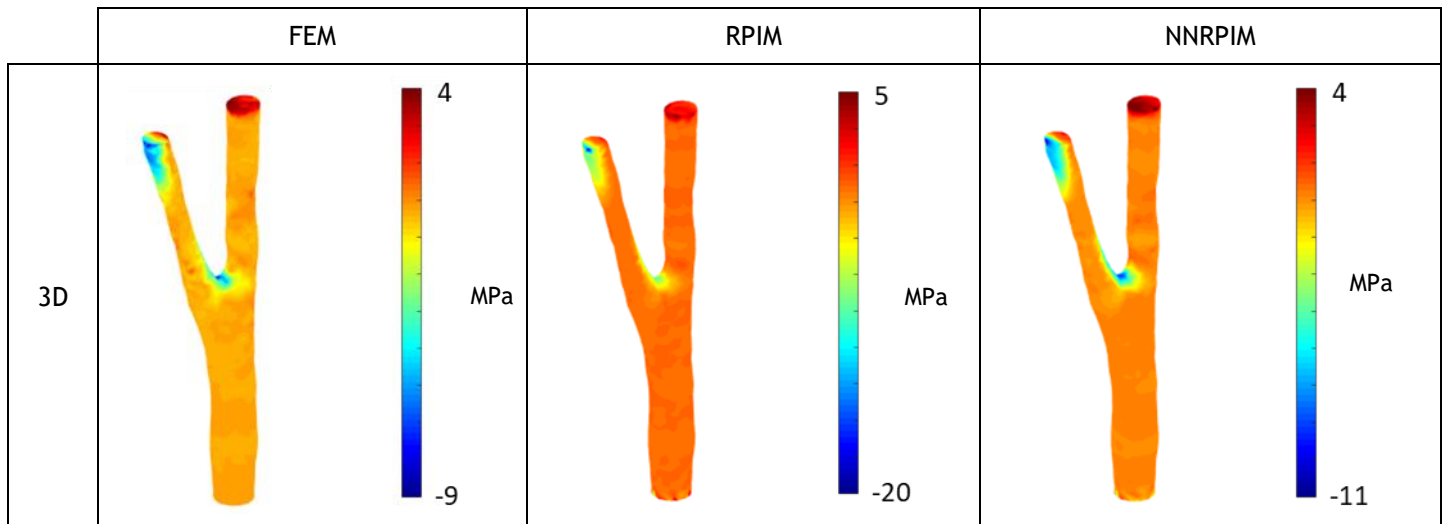


Table 11 - σ_{33} stress of 3D model.



In this study, it was analyzed the stresses of a peripheral nerve bifurcation when an external force of 0.423 N is stretching the nerve. This numerical experiment was performed using the boundary conditions mentioned before. The result of the stress distribution in the model is expressed in color maps in a color gradient that varies between red and blue, represented by

the red color the highest tension values recorded. For the validation of both models, there was a comparison between the axial displacements of them. The total displacement results for the selected points in the two models are very similar; this corroborates the efficiency of the methods used. The results obtained for the von Mises effective von Mises stress are concordant, however, the visible difference in values can be justified by several factors, such as the difference between the geometry of the two models (i.e., size and shape); the area of application of the load and the differences in the boundary conditions. The main stress is always observed in the same region in both models (for all the three methods), that stresses did not exceed 11 MPa,

Regarding the versatility of the numerical model, it was verified that it allows an overall evaluation of the behavior of the peripheral nerve, although the results concordant, to increase the level of accuracy and precision of the same, it would be necessary to increase the number of elements and nodes.

Chapter 7

Elasto-static numerical analysis

In this chapter are presented several numerical tests of chitosan tubes models used as NGC's, using three distinct numerical methods: FEM, RPIM and NNRPIM. As previously mentioned, chitosan NGC's can be an alternative solution to the autologous nerve grafts, but it must be guaranteeing its mechanical resistance, to obtain a suitable and functional recovery procedure. Therefore, it will be presented all the elasto-static numerical analysis performed.

Initially, a 3D model was developed with the main objective of performing a convergence study, where it was found the best nodal discretization for the geometric model with a good computational cost-benefit ratio. Moreover, the three numerical methods that were used (FEM, RPIM and NNRPIM), were compared in order to understand the level of performance of meshless methods.

Regarding the FEM, for the three-dimensional analyzes, it was considered tetrahedral elements of four nodes (FEM-4n). Concerning RPIM formulation, the RPI shape functions were constructed using the MQ-RBF and a linear polynomial basis. There were considered 27 nodes within the domain of influence, $c = 0.0001$ and $p = 0.9999$. For the 3D analysis using NNRPIM, the following parameters were considered: The nodal connectivity is imposed using the second-degree influence-cells, $c = 0.0001$, $p = 0.9999$ and constant polynomial basis. The integration points are obtained with the low order integration scheme.

After the convergence study, more analyses were performed using the nodal discretization obtained by the previously mentioned study, where the geometry of the chitosan tube was considered, varying several factors such as nerve gap, suture distance, tube thickness and tube external geometry.

All the models were constructed in FEMAP[®] and all the structural analysis was performed in FEMAS[®] (Finite Element and Meshless Analysis Software - cmech.webs.com) - a freeware academic program which allows the structural and mechanical analysis of computational models.

7.1- Study of convergence

Nodal discretization is one of the significant factors that can influence results in numerical analysis. Therefore, a convergence study was first carried out to find the best nodal discretization for the geometric models, considering the computational cost-benefit ratio. The dimensions of the 3D geometric model 1 can be observed in Figure 48.(a). The model was composed of one single patch Figure 48.(b).

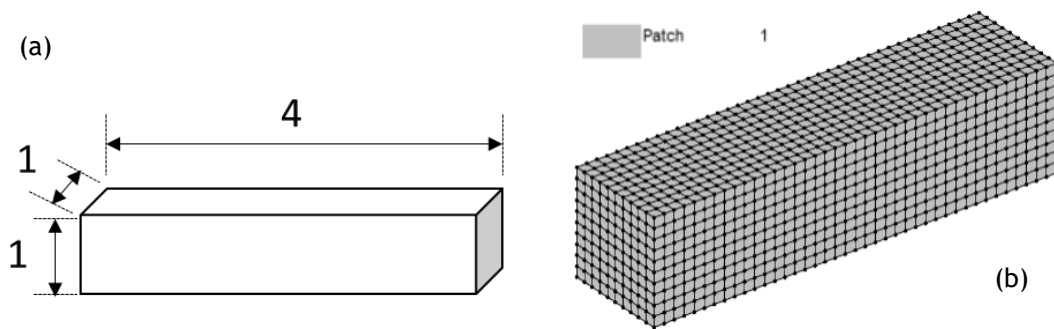


Figure 48 - (a) Dimensions of the model 1 in millimetres (mm); (b) model 1 patch.

The number of nodes that the patch should have was obtained from the length of the curves that outlined it, being increased in proportion until reaching a more uniform mesh. For geometric model 1, six meshes were created. In the construction of the 3D models with an increasing number of nodes, a geometric progression was applied to each section. Due to the reduced computational power available for this study, it was not possible to construct models with an even more significant number of nodes. The following table (Table 12) shows the nodal density in the different six models.

Table 12 - Different nodal density used in model 1 on the convergence study.

Model 1	nodes
(a)	81
(b)	425
(c)	1225
(d)	2673
(e)	4961
(f)	8281

The material was considered homogeneous, linear elastic and isotropic and their mechanical properties are indicated in Table 13.

Table 13 - Mechanical proprieties of the material.

model	Young Modulus (E) [Mpa]	Poisson Ratio (ν)
1	1	0.3

For the case studied, the boundary conditions considered were applied to one of the small sides of the model; these conditions are represented in Figure 49. For the stress analysis, a traction load was applied in the model. It was applied 1 N of force in the direction of the y-axis and distributed along the section, represented in Figure 4, in order to perform a flexion study.

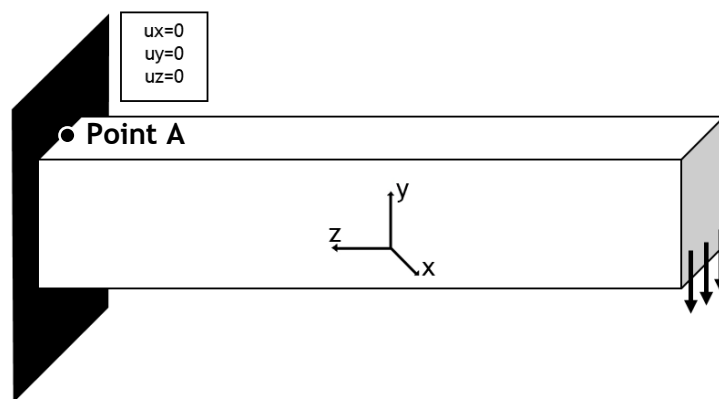


Figure 49 - Boundary conditions and forces applied in the 3D model 1.

Selecting on the surface where the forces were applied the central point, it was possible to ascertain the displacement obtained with the different nodal densities and the three numerical methods, FEM, RPIM and NNRPIM. However, it is essential to note that the selected node was always located in the same position. The results obtained are shown in Figure 50.

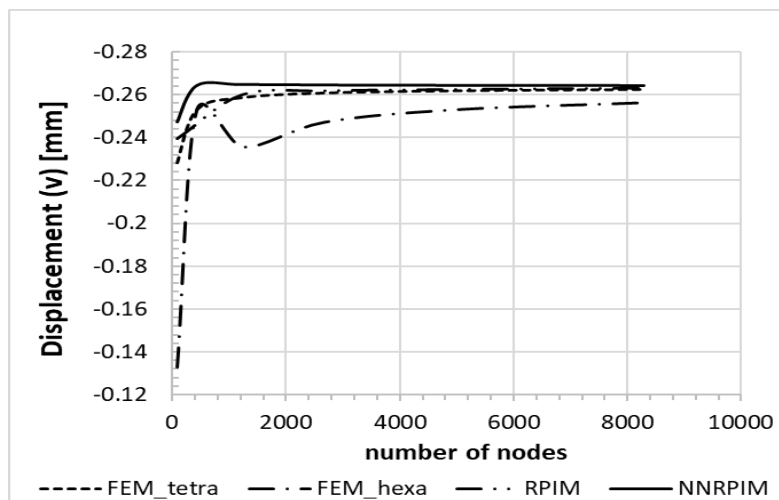


Figure 50 - Total displacement as a function of the nodal discretization of the 3D geometric model.

The obtained values indicate that the methods converged. It is known that with the increase in number of nodes the more refined the meshes become and the more precise the solutions are; however, the results are already very close to the final converged value (all lines are almost flat). Thus, it is possible to infer that a nodal discretization with about 5000 nodes allows the analysis to be carried out with a satisfactory computational cost.

Two points (A and B) were selected to analyze the stress (σ_{xx}), these points are located on a side surface of the model as Figure 49 shows, the point B is vertically opposite to point A. The obtained results are represented in Figure 51.

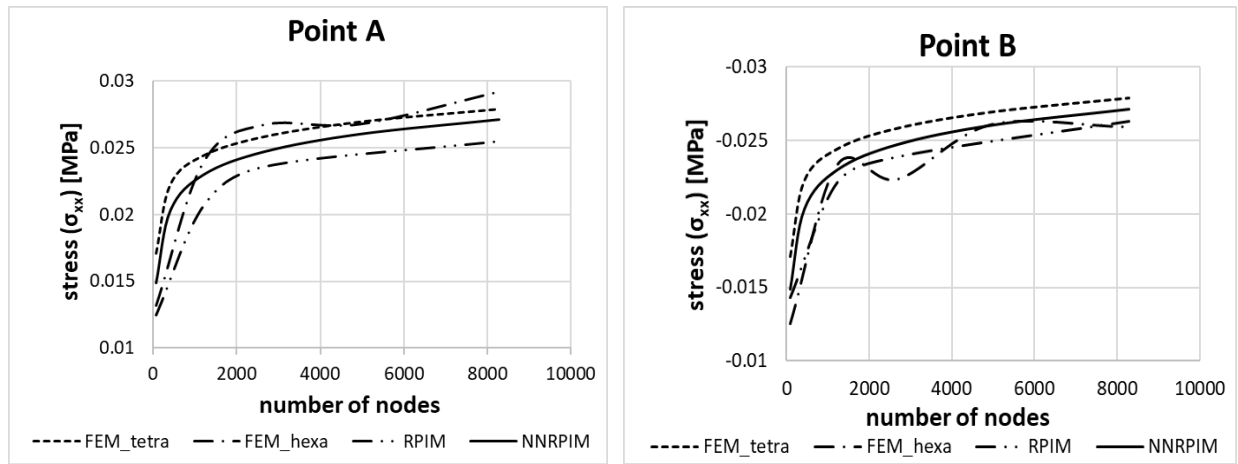


Figure 51 - Stress (σ_{xx}) on point A and B as a function of the nodal discretization of the 3D geometric model 1.

The obtained results indicate the convergence of the methods. However, there are slightly differences between them. These differences are affected by the nodal density. The meshless methods demonstrate that they are an effective alternative to the conventional method (FEM). Comparing the meshless analysis results (RPIM and NNRPIM) with the FEM analysis results, it is possible to see the convergence of the meshless methods without the oscillation obtained for FEM-hexa (Figure 51), which can demonstrate the effective performance of this innovative methods.

A second convergence study was done using model 2, which was adapted from a real chitosan NGC, a patented chitosan tube by REAXON® for nerve regeneration [99]. The dimensions of the tube were projected for sciatic nerve regeneration.

This dimensions of the 3D geometric model 1 can be observed in Figure 52(a) The model was composed of three different patches, the first patch consists in the chitosan tube section where the peripheral nerve is sutured, the second corresponds to the peripheral nerve and the last patch consists in the section of the chitosan tube in the nerve gap Figure 52(b). The model of the chitosan tube is composed of half of the tube. Thus, the nerve gap corresponds to the half of the real nerve gap; this was done due to the reduce computer power available to perform this study.

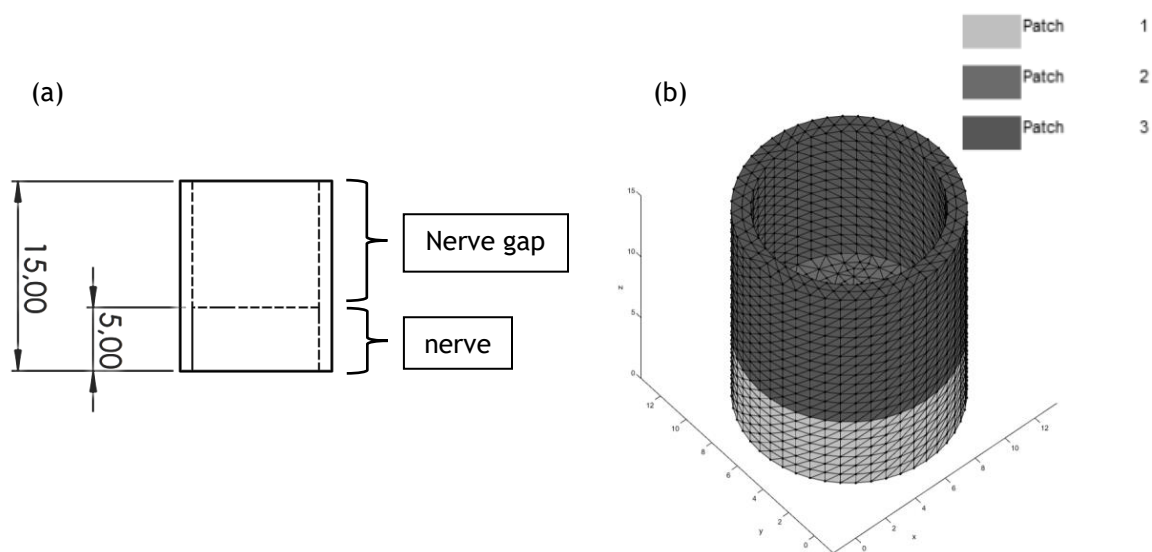


Figure 52 - (a) Dimensions of the chitosan tube in millimetres (mm); (b) chitosan tube model 2 with the three different patches.

The number of nodes that each patch should have was obtained from the length of the curves that outlined it, being increased in proportion until reaching a more uniform mesh. For the geometric model 1, seven meshes were created, which can be observed in Figure 53, the model with the smaller number of nodes and the model with the significant number of nodes.

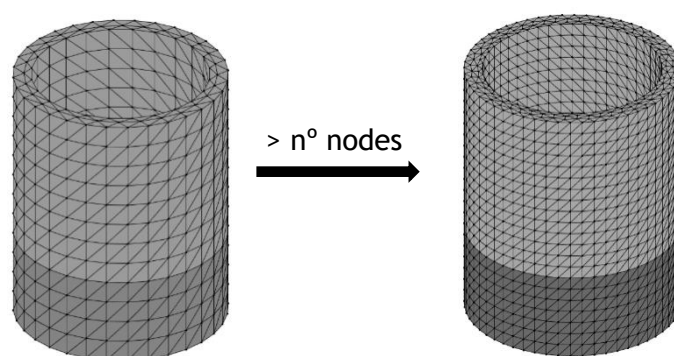


Figure 53 - Model 2 with smaller (left) and larger (right) number of nodes, used in the convergence study.

In the construction of the 3D models with increasing number of nodes, in a first phase it was constructed a model where each millimeter of the curves that outlined the model corresponded to a division, after that first model to construct the following were used several multiplicative factors (x0.75, x0.875, x1.15, x1.25, x1.3, x1.4) applied to each section. Due to the reduced computational power available for this study, it was not possible to construct

models with an even greater number of nodes. The following table (Table 14) shows the nodal density in the different seven models.

Table 14 - Different nodal density in the models used in convergence study.

Model 2	nodes	elements
(a)	1337	1824
(b)	1878	2652
(c)	2214	3120
(d)	3355	4848
(e)	4060	5904
(f)	4216	6040
(g)	5928	7056

All materials were considered homogeneous, linear elastic and isotropic and their mechanical properties are indicated in Table 15.

Table 15 - Mechanical proprieties of the materials.

Material	Young Modulus (E) [Mpa]	Poisson's Ratio (ν)	Literature Ref.
chitosan tube	7.06	0.4999	[63], [65]
sciatic peripheral nerve	40.96	0.37	[44]

For the case studied, the boundary conditions considered were applied on the top of the chitosan tube, these conditions are represented in Figure 54.

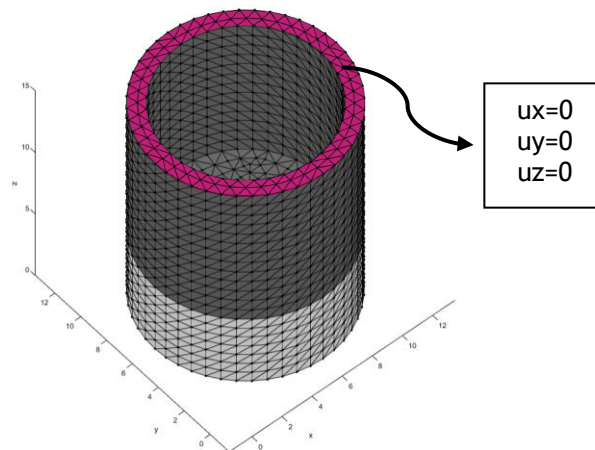


Figure 54 - Boundary conditions imposed on model 2.

For the stress analysis, a triangular load was applied in one end of the tube, Figure 55. The load aims to induce a known bending moment. The maximum value of the triangular distributed load is 1 N/m, applied in the Z-axis direction.

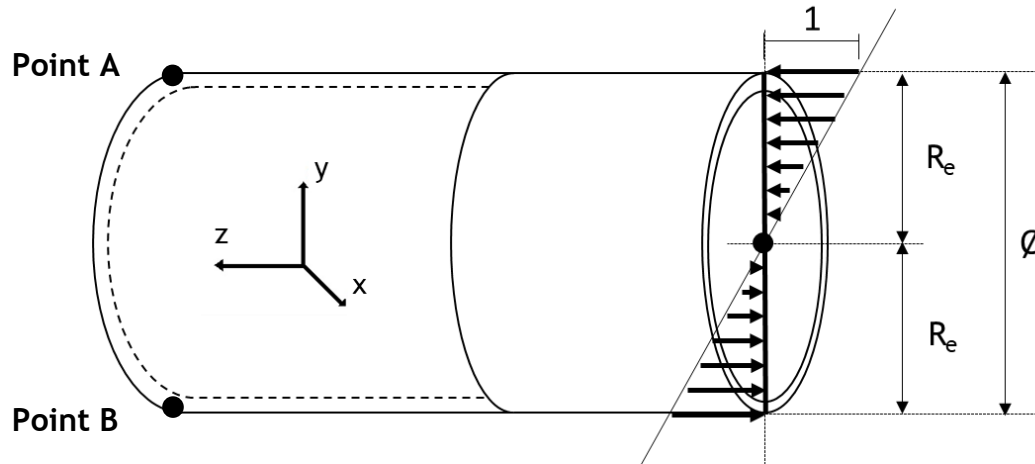


Figure 55 - Forces applied in the 3D models.

The applied moment (M) can be calculated by the following equation (7.1). The M applied to the model is of 24 Nm.

$$M_{applied} = R_e \times F + R_i \times F \quad (7.1)$$

The applied forces were distributed along the represented cross section in the direction of the Z-axis according to the following equation

$$f(x) = a_0 + a_1 x \quad (7.2)$$

Where $a_0 = 1$ and $a_1 = \frac{-2}{\delta}$.

Selecting on the surface where the forces were applied the central point, it was possible to ascertain the displacement obtained for the different nodal densities and the three numerical methods, FEM, RPIM and NNRPIM. However, it is essential to note that the selected node was always located in the same position. The results obtained are shown in Figure 56.

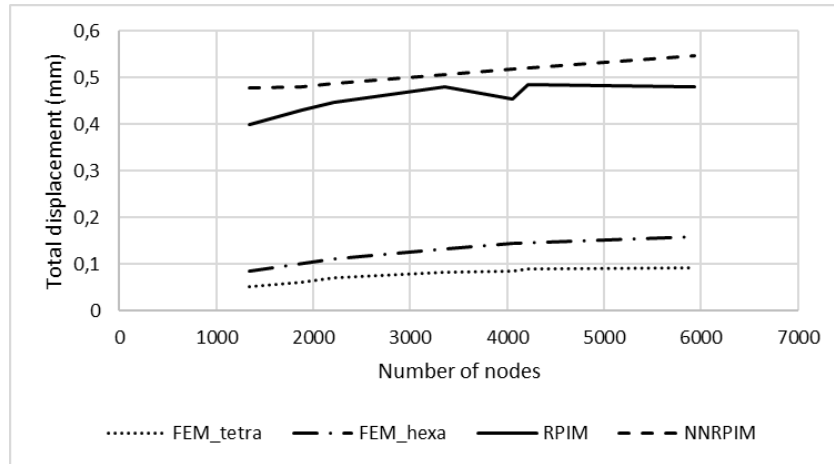


Figure 56 - Total displacement as a function of the nodal discretization of the 3D geometric model.

Using FEM, the obtained values are smaller when compared to the two methods without mesh. It is known that with the increase in number of nodes we get more refined meshes and more precise solutions. However, the results are already very close to the final converged value (all lines are almost flat) between the two meshless methods. The difference between the FEM and the meshless values can be justified by the low node density due to the small computational power available. Thus, it is possible to infer that a nodal discretization with about 5000 nodes allows the analysis to be carried out with the available computational power.

Two points (A and B) were selected to analyze the effective von Mises stress (σ_{ef}), these points are located on the top surface of the tube as figure 8.4 shows. The obtained results are represented in Figure 57.

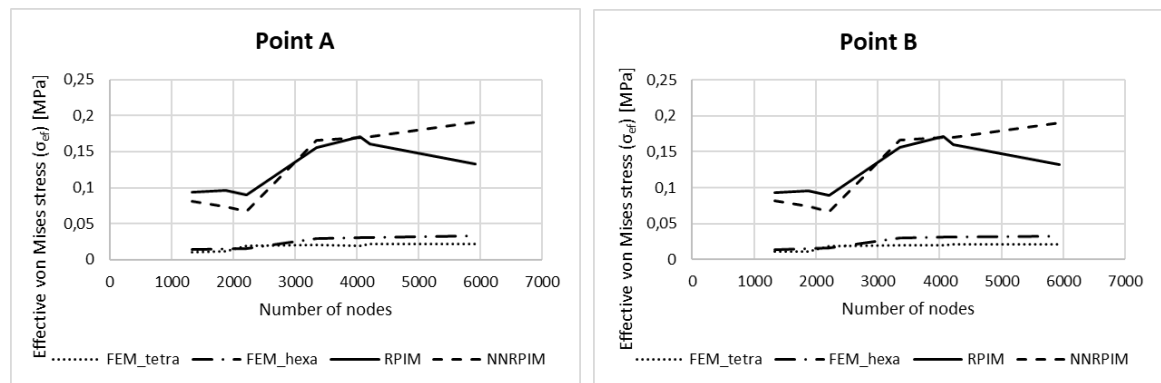


Figure 57 - Effective von Mises stress on point A and B as a function of the nodal discretization of the 3D geometric model.

The following tables (Table 16 and Table 17) shows isomaps of the different results obtained with the three distinct numerical methods for all the seven different chitosan tube models, including the effective von Mises stress, σ_{11} and σ_{33} .

Table 16 - Results obtained in FEM and RPIM structural analysis in the second convergence study for effective von Mises stress, σ_{11} and σ_{33} .

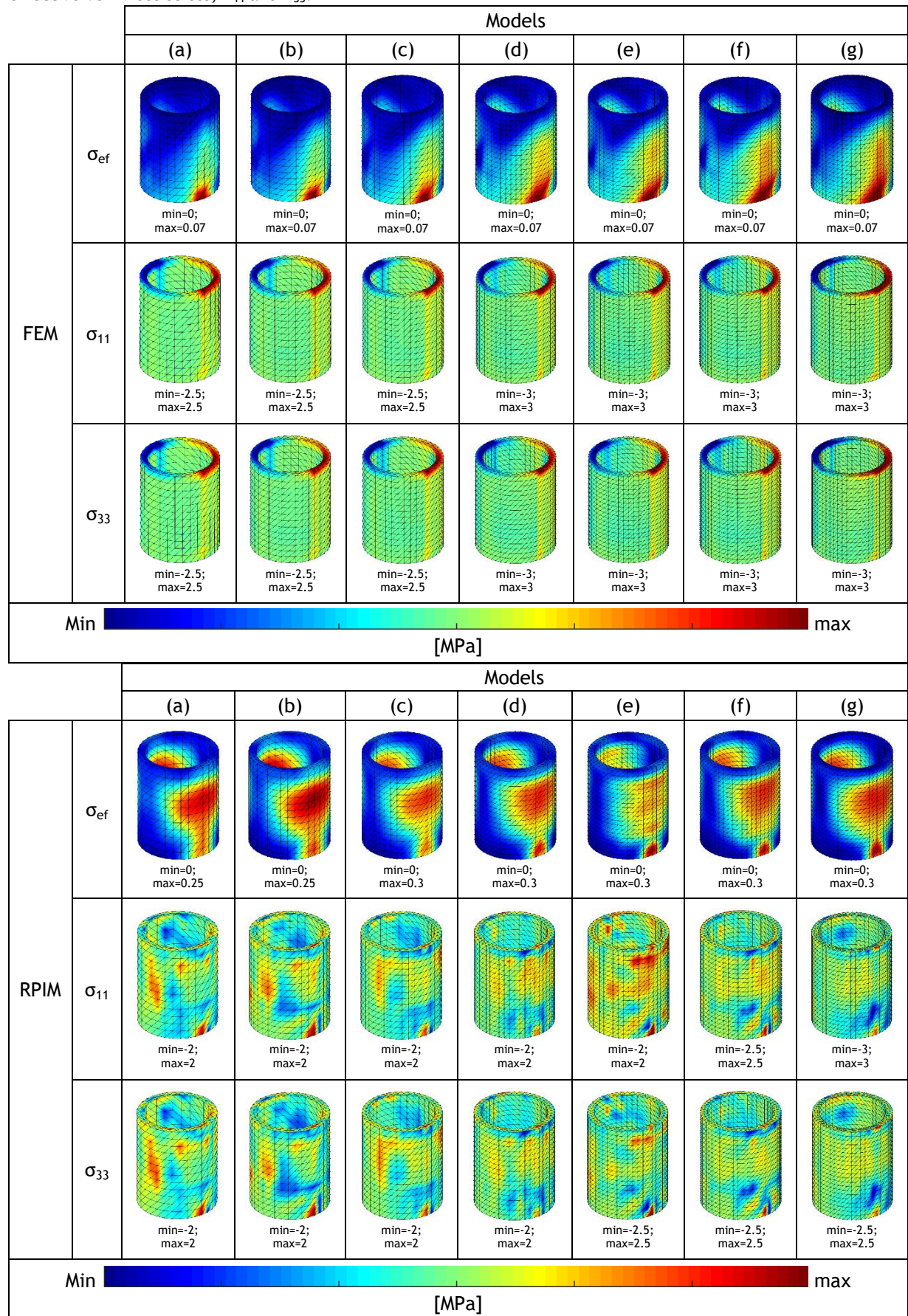


Table 17 - Results obtained in NNRPIM structural analysis in the second convergence study for effective von Mises stress, σ_{11} and σ_{33} .

		Models							
		(a)	(b)	(c)	(d)	(e)	(f)	(g)	
NNRPIM	σ_{ef}	 min=0; max=0.3	 min=0; max=0.3	 min=0; max=0.3	 min=0; max=0.3	 min=0; max=0.3	 min=0; max=0.3	 min=0; max=0.3	
	σ_{11}	 min=-2.5; max=2.5	-2 - 2	-2 - 2	 min=-2.5; max=2.5	 min=-2.5; max=2.5	-3 - 3	-3 - 3	
	σ_{33}	 min=-2.5; max=2.5	-2 - 2	-2 - 2	 min=-2.5; max=2.5	 min=-2.5; max=2.5	 min=-2.5; max=2.5	-3 - 3	
Min									max
		[MPa]							

The obtained results in the first converge study indicate the convergence of the methods and demonstrate that the meshless are effectively capable of performing structural analysis as the FEM, being a viable alternative. In the second convergence study, the convergence of the methods was not reached due to the limited computational power available, because the vast dimensions of the models demand a higher nodal density. However, a high nodal density increases the needed for powerful computation power. Relating the meshless analysis results (RPIM and NNRPIM), it is possible to see the convergence of the meshless methods between them in the second study.

Due to computation power limitations, a nodal density of 5000 nodes will be used in the following studies, which assures a good computational cost-benefit ratio; however, for more accurate results, a higher nodal density is needed.

7.2 - Study of chitosan tube geometry

It is essential to study the internal geometry of the tube in order to determine the ideal dimensions of the chitosan tube for a better process of peripheral nerve regeneration. In the

present study, three different aspects of the tube geometry were studied: nerve suture distance, nerve gap distance and chitosan tube thickness. The nerve suture dimensions considered were: 5, 10, 15, 20, 25 and 30 millimetres (mm); the nerve gap distances were: 2.5, 5, 7.25 and 10 millimetres (mm); three different tube wall thickness were considered: 1, 2 and 2.5 millimetres (mm). Thus, 72 different models were constructed varied the aspects previously mentioned with an average nodal distribution of 5000 nodes due to reduced computational power available for the analysis (Figure 58).

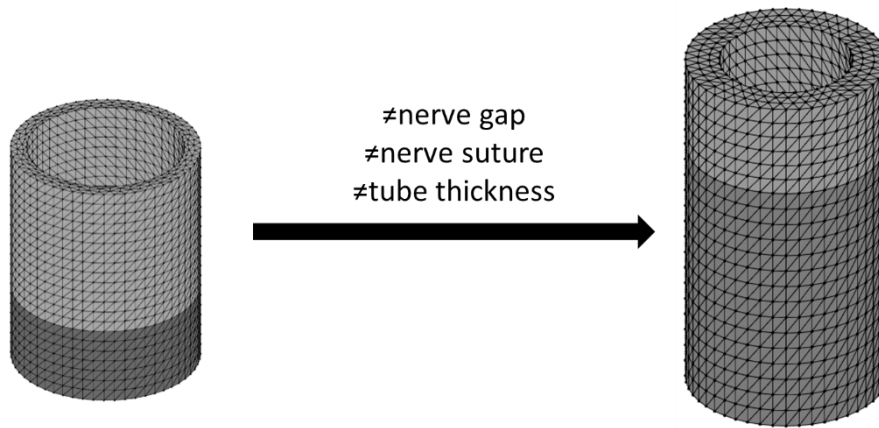
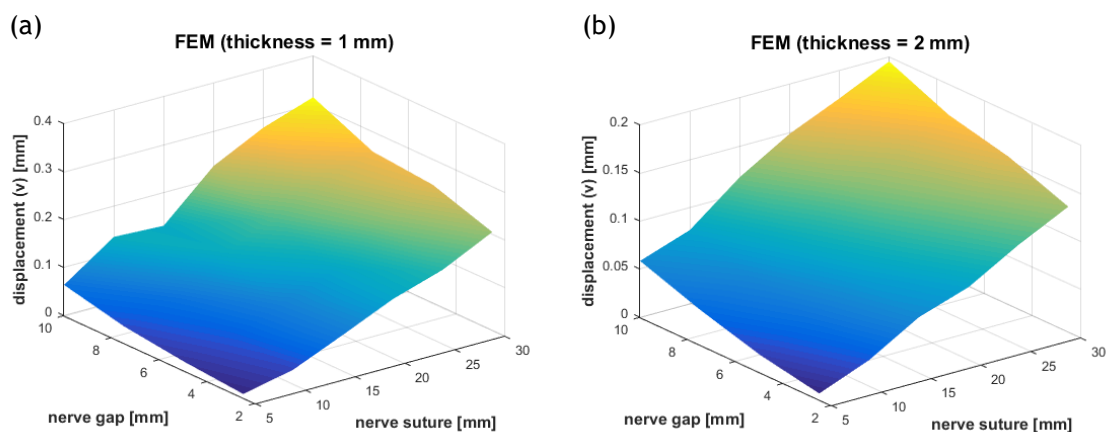


Figure 58 - schematic of the construction of the different chitosan tube models.

The models were subjects to the same stress analyses that model 2 in section 8.1.1 was submitted. For the stress analysis, the same triangular force described in Figure 55 was applied in the opposite extremities of the chitosan tube where the peripheral nerve is sutured and distributed along the cross-section. The boundary conditions were the same mentioned in the previous section for model 2 ($u_x=0$; $u_y=0$; $u_z=0$) on the top extremity of nerve gap. The displacement (v) and the effective von Mises stress were analysed using two different advanced numerical methods: FEM and RPIM. The points were the displacement (v), and the effective von Mises stress were analysed are the same as in the convergence study model 2, point A and B on top extremities of the nerve gap for stress analysis and the central point at the nerve suture for displacement analysis (bottom of chitosan tube). After completing the numerical tests, with the results obtained it was possible to construct several 3D graphs that allow to see how the different chitosan tubes behave with their varied geometry (thickness, nerve suture length and nerve gap length).

Figure 59 shows the results for the displacement (v) for all the different models.



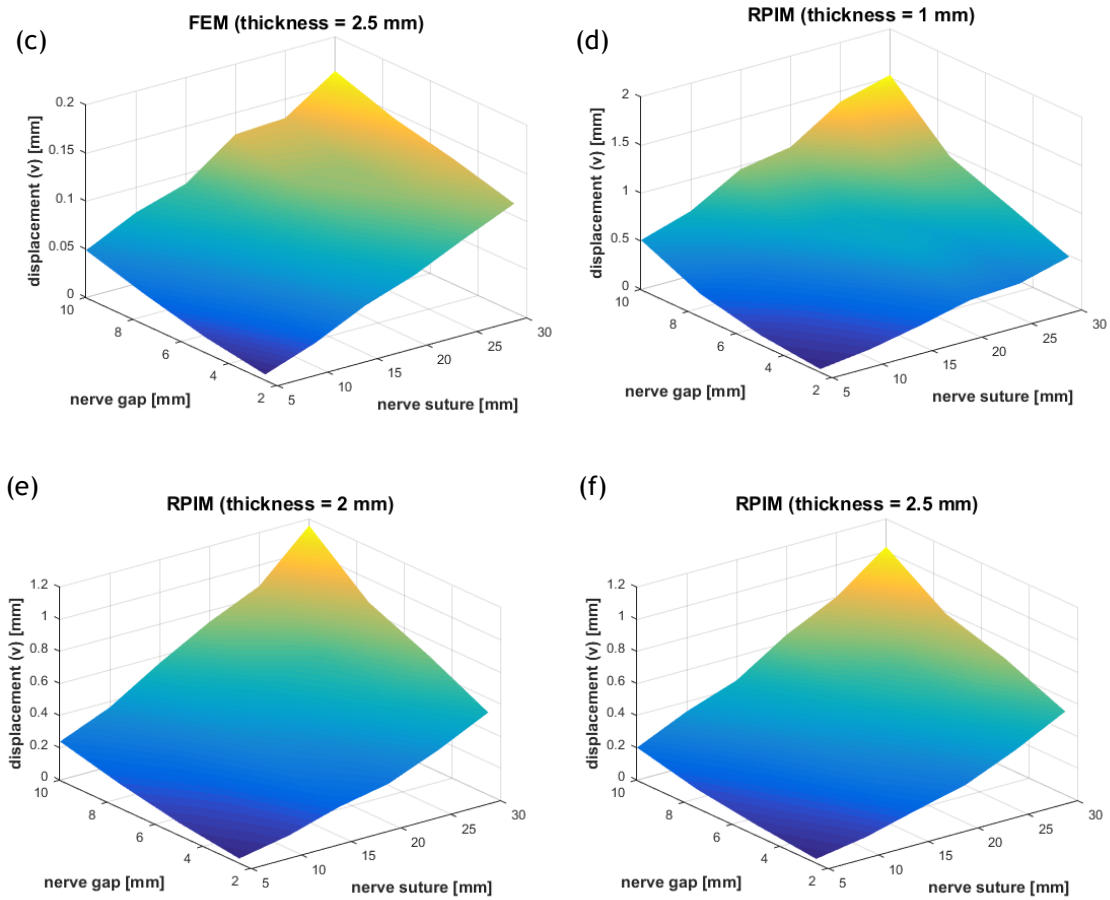


Figure 59 - (a),(b),(c) - FEM results for displacement (v) vs nerve gap and nerve suture lengths. (d),(e),(f) - RPIM results for displacement (v) vs nerve gap and nerve suture lengths.

Figure 59 shows the relationship between the increase of the nerve gap length and the nerve suture length. In Figure 59 (a) the chitosan tube has a thickness of 1 millimeter, the increase of the nerve gap length increases displacement (v), the increase of the nerve suture length also increases the displacement (v) of the chitosan tube. Thus, these two parameters have a similar influence in the displacement of the tube.

Thus, it is possible to infer that a change in the nerve suture length has almost an equal influence in the displacement of the chitosan tube as a change in the nerve gap length. In Figure 59 (b) and (c) the behaviour of the tubes are similar to Figure 59 (a) but with the increase of the tube wall thickness the values of the displacement decreases which means that the thickness of the chitosan tube has a direct influence on the mechanical behaviour of the chitosan tube, decreasing the displacement (v) when the thickness of the tube increases. The chitosan tube model with the smallest nerve gap length (2.5mm) and with the smallest nerve suture length (5 mm) had a small displacement (v), and the tube with the bigger nerve gap a suture length had the biggest displacement (v).

The analysis of the displacement using RPIM allow to obtain identical results to the FEM analysis, the Figure 59 (a) 3D graph is similar to (d), (b) to (e) and (c) to (f). However, there are differences in the order of magnitude of the values. These differences can be justified due to the small density in nodes of the models, due to the reduced computational power available

the models had to be constructed with a small number of nodes which cannot ensure the convergence of this meshless method. In order to get a reduced difference between the values of displacement, the models must have a higher number of nodes.

Figure 60 shows the results for the effective von Mises stress for all the different models using FEM and RPIM.

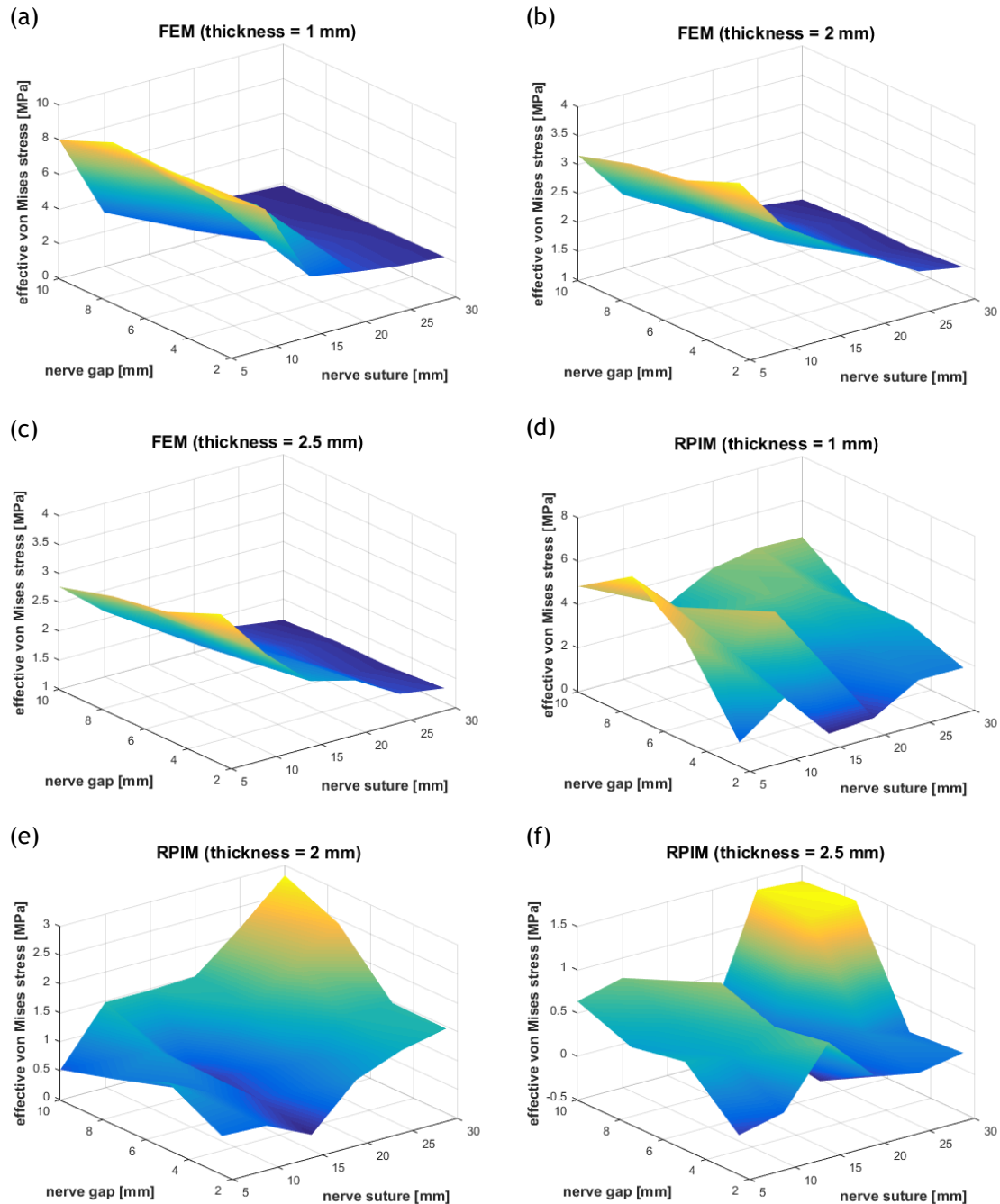


Figure 60 - (a),(b),(c) - FEM results for effective von Mises stress vs nerve gap and nerve suture lengths. (d),(e),(f) - RPIM results for effective von Mises stress vs nerve gap and nerve suture lengths.

The 3D graphs in Figure 60 show that the increase of the nerve gap length results in the decrease of effective von Mises stress and the increase of the nerve suture length also decreases the effective von Mises stress of the chitosan tube. When compared with Figure 59, the thickness of the chitosan tube wall also had a great influence on the values of the effective von Mises stress. With the increase of the wall thickness, the effective von Mises stress obtained in the top points (A and B) of the chitosan tube reduces. The RPIM analysis 3D graphics (e) and (f) shows some unexpected values that can be justified, as it been mentioned before, due to the small density in nodes of the models. The results comparing the increasing of the chitosan wall thickness agree with the FEM results, for higher values of thickness, the values of effective von Mises stress are lower. To more accurately evaluate the effect of the thickness in the displacement and effective von Mises stress obtained in the chitosan tube, the Figure 61 and Figure 62 shows the graphs of Figure 59 and Figure 60 assembled, where the thickness is in function of nerve gap or nerve suture. The Figure 61 shows the FEM results.

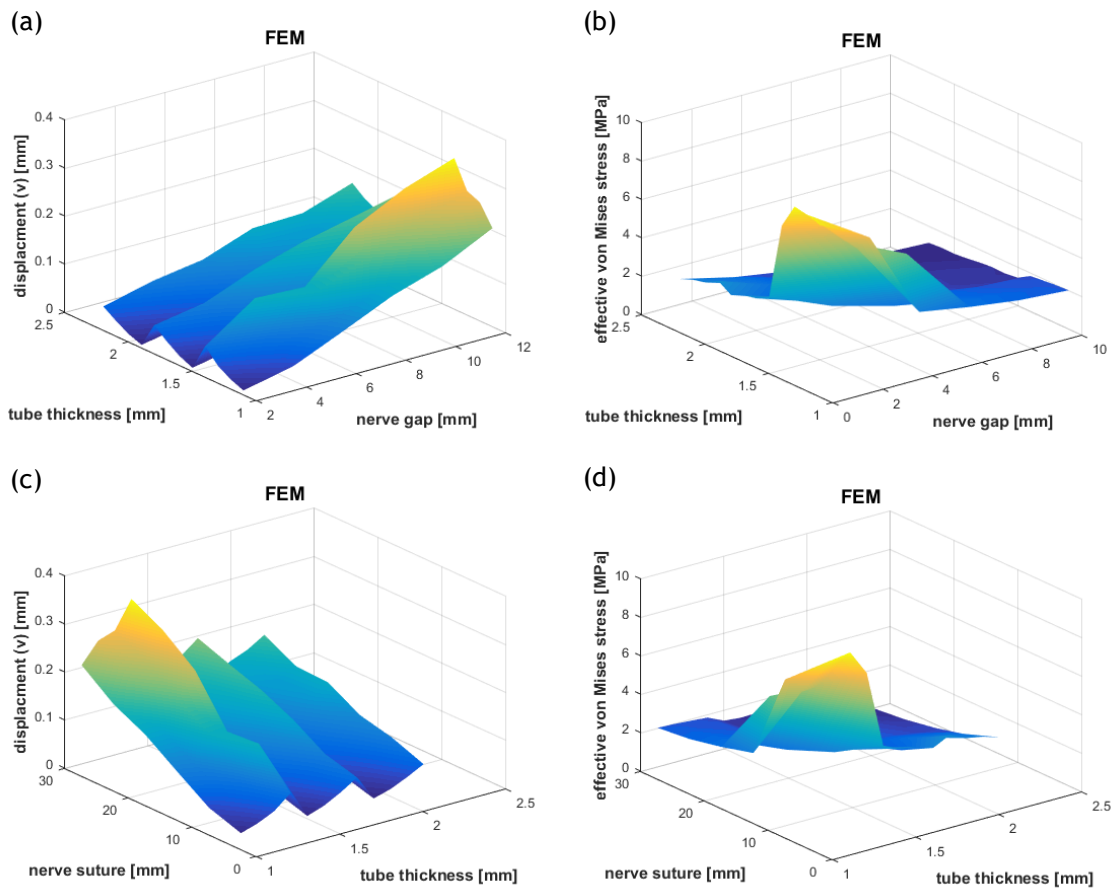


Figure 61 - FEM results for displacement (v) ((a) and (c)) and effective von Mises stress ((b) and (d)) for the different thickness vs nerve gap or nerve suture lengths.

The results showed in Figure 61 are concordant with the previous mentioned, the thickness of the chitosan tube wall affects the displacement and the effective von Mises stress. Thus, when the thickness increases the displacement and the effective von Mises stress decreases. In Figure 61 (b) and (d) it is possible to see a marked decrease in the effective von Mises stress when the nerve suture is around 20 mm, and the tube thickness around 2 mm.

Figure 62 shows the RPIM results for displacement (v) and effective von Mises stress for the different thickness vs nerve gap or nerve suture lengths.

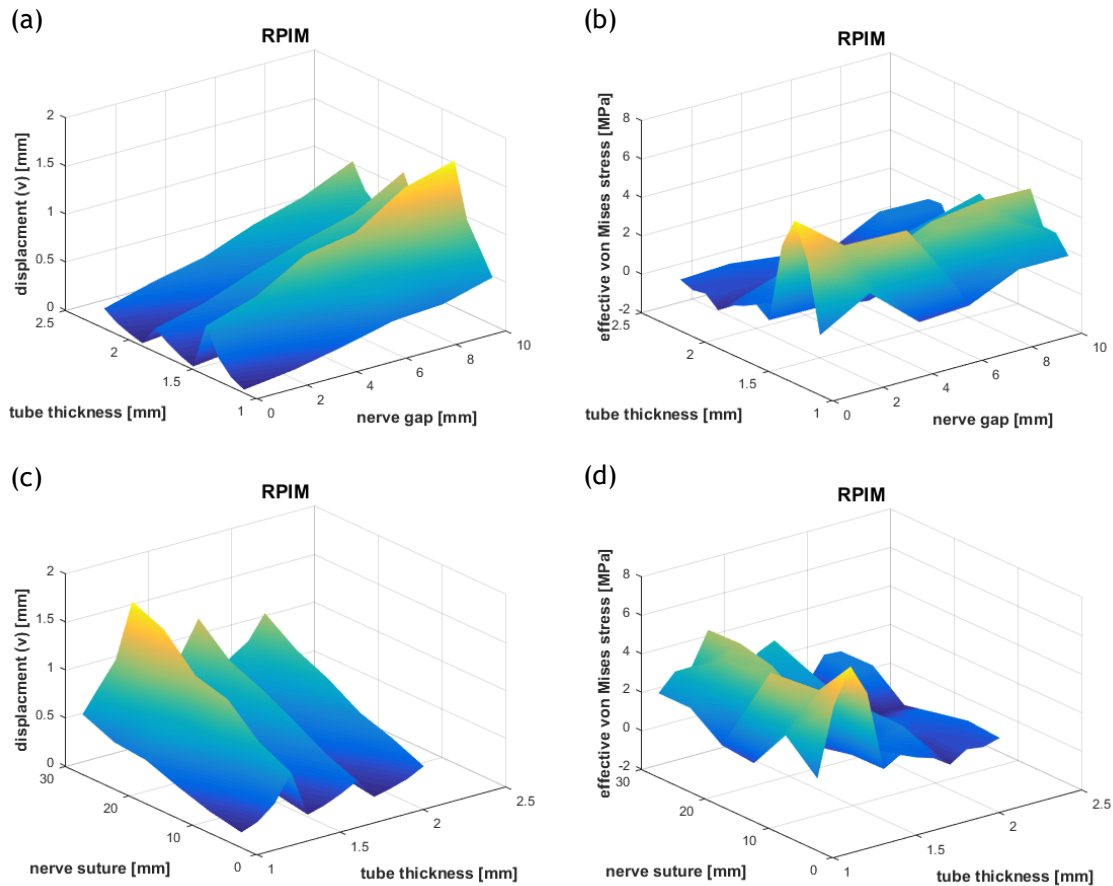


Figure 62 - RPIM results for displacement (v) ((a) and (c)) and effective von Mises stress ((b) and (d)) for the different thickness vs nerve gap or nerve suture lengths.

The analysis of the displacement using RPIM allows obtaining identical results to the FEM analysis. Through these 3D graphs, it becomes easier to visualize the influence of thickness on the biomechanical behaviour of the model. As in FEM analysis, there is a marked decrease in the values of effective von Mises stress when the tube thickness of the chitosan wall is 2 mm, which means that with higher values of thickness it occurs a reduction of the effective von Mises stress obtained in the top points of the chitosan tube. Thus, the nerve inside the tube will experiences less mechanical stress for these higher values of thickness. However, the values of displacement also decrease with the increase of thickness. The decrease in displacement affects the mobility of the recovering patient. Thus, the best tube will be the one that can withstand greater stress and with controlled freedom of displacement. The tube

with a nerve suture of 20mm and 10 mm of nerve gap will be a good choice for a reparative procedure of patient with a nerve lesion, but there are other factors to take in account such as the real dimension of the nerve gap in the patient.

In order to estimate the value of displacement for the present values and other values of nerve gap and nerve suture, it was created a function with different variables which try to approximate to the real values of the displacement. This function is represented in the following equation (7.3).

$$displacement = \mu \times (\alpha \times suture^{\beta}) \times (\gamma \times gap^{\zeta}) \quad (7.3)$$

Thus, through the equation previously presented it is possible to construct the following 3D graphs (Figure 63) where the original 3D graph for displacement is represented, and the new approximation graph is also represented with greater transparency.

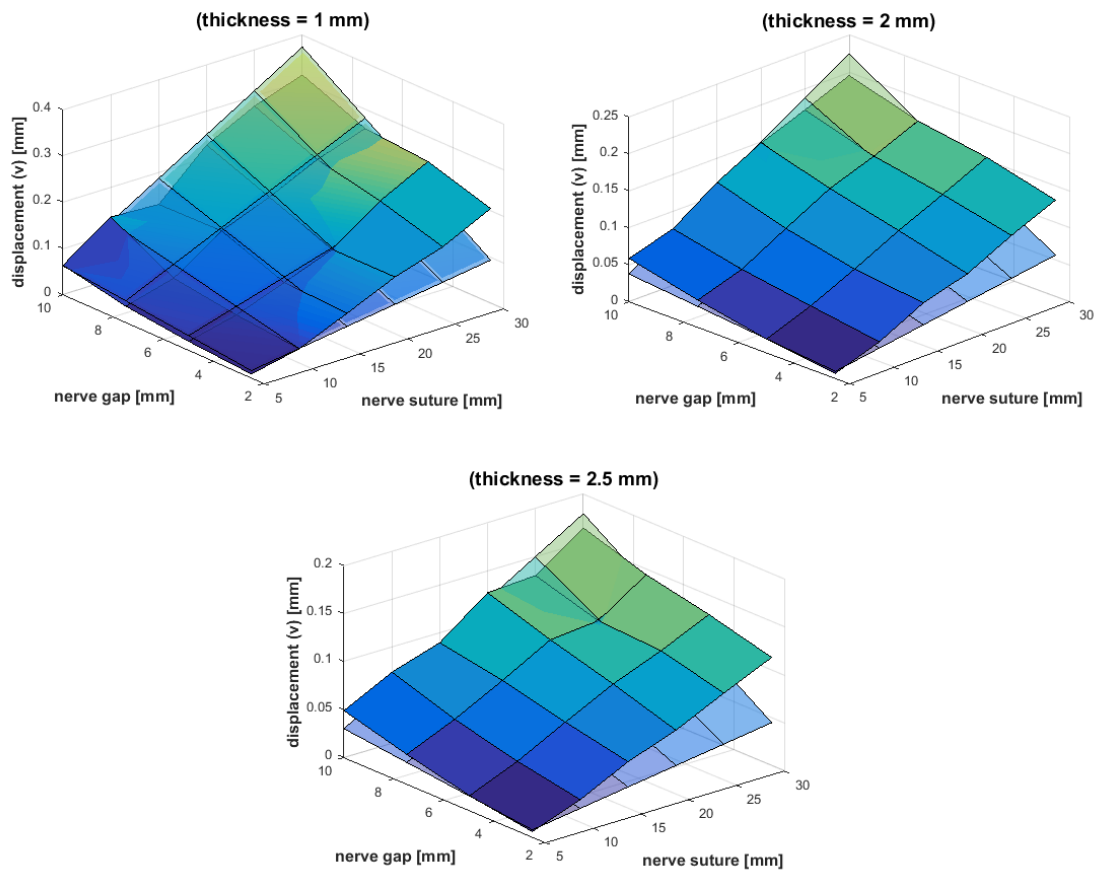


Figure 63 - Approximation functions (more transparent) for displacement (v) for the three different thickness of the chitosan tube wall.

The presented approximation functions can estimate with a good level of accuracy the values of displacement of the chitosan tubes with different values of nerve suture, nerve gap and thickness of the tube wall. The following Table 18 shows the values of the parameters used in the approximation function used for the construction of the 3D graphs.

Table 18 - Values of the parameters used in the approximation functions of displacement (v).

<i>Thickness [mm]</i>	μ	α	β	γ	ζ
1	1	0.005	1	0.25	1
2	1	0.0075	1	0.1	1
2.5	1	0.006	1	0.1	1

7.3 - Study of chitosan tube external geometry

The external geometry of the chitosan tube has a great impact on the mechanical behaviour of the tube. In this study, seven different models of the chitosan tube were constructed, using the ideal dimensions mentioned in the previous section, which are 20 mm for the nerve suture length and 10 mm for nerve gap length. The thickness of the tube wall on the bottom (nerve suture site) is fixed in 1mm and the on the top it varies between 1, 1.25, 1.5, 1.75, 2, 2.25 and 2.5 mm (Figure 64). On the construction of the models, it was used a spline between the extremities.

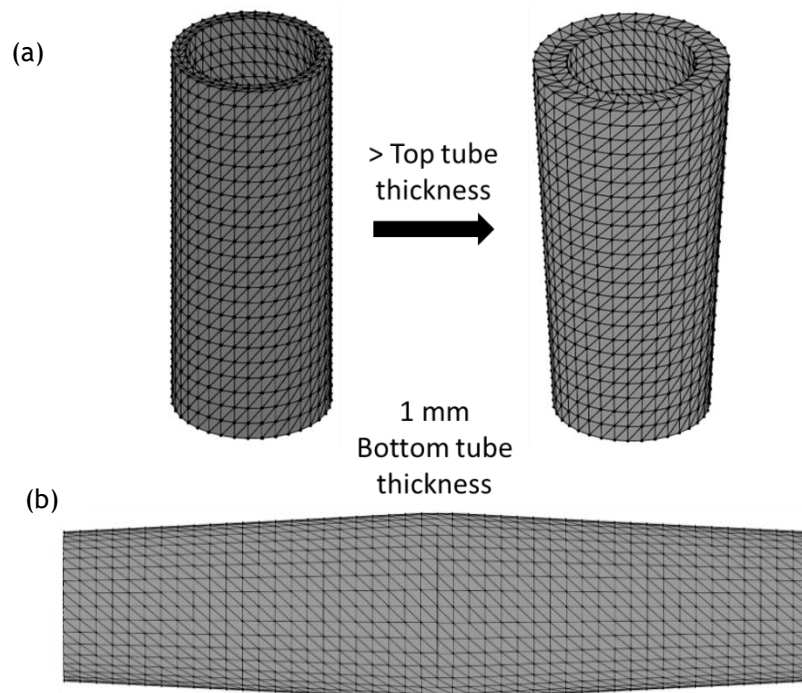


Figure 64 - (a) schematic of the construction of the chitosan tube models. (b) The appearance of the total chitosan tube.

The models were submitted to the same stress analyses that models in the previous section 8.1.2 were submitted to. Thus, the same triangular force described in Figure 55 was applied in the opposite extremities of the chitosan tube where the peripheral nerve is sutured and distributed along the cross-section. The boundary conditions were the same mentioned in the previous section ($u_x=0$; $u_y=0$; $u_z=0$) on the top extremity of nerve gap. Thus, seven different models were constructed varied the aspect previously mentioned with an average nodal distribution of 5000 nodes due to reduced computational power available for the analysis.

The total displacement ($|u|$) and the effective von Mises stress were analysed using two different advanced numerical methods: FEM and RPIM. The points were the total displacement ($|u|$), and the effective von Mises stress were analysed are the same has in the previous section, point A and B on top extremities of the nerve gap for stress analysis and the central point at the nerve suture for displacement analysis (bottom of chitosan tube), this way it will be possible to compare the results with the previous section.

Figure 65 shows the obtained results by FEM for the effective von Mises stress when the tube thickness changes.

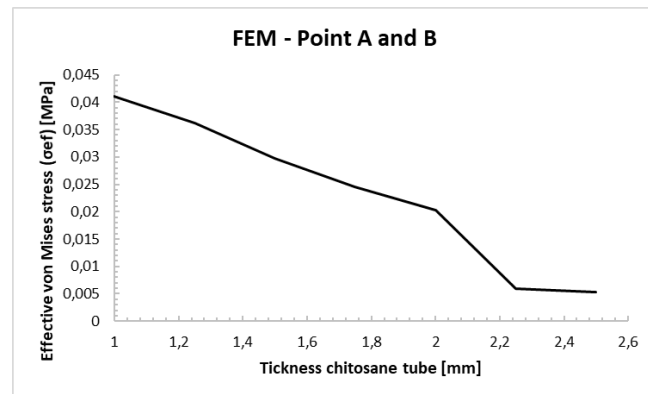


Figure 65 -FEM results for effective von Mises stress in function of the different thickness in the top of the chitosan tube.

The FEM results show that the effective von Mises stress decreases when the tube wall thickness increases; it is almost a linear decrease. Figure 66 shows the obtained results by RPIM for the effective von Mises stress when the tube thickness changes.

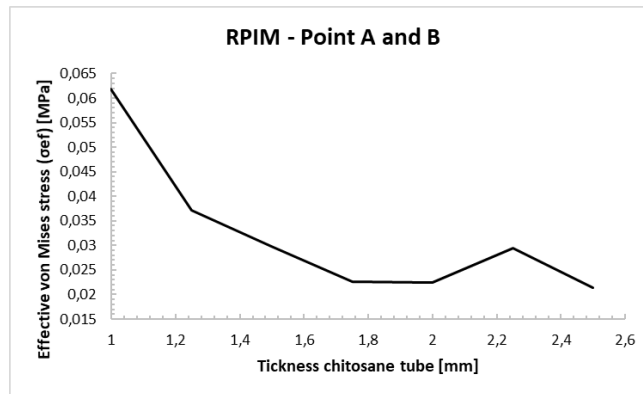


Figure 66 -RPIM results for effective von Mises stress in function of the different thickness in the top of the chitosan tube.

The RPIM results agree with the FEM results; the effective von Mises stress decreases when the tube thickness wall increases. The decreasing of the effective von Mises stress in RPIM is lower after 1.5 mm of thickness when compared with FEM. The FEM presents a more linear result when compared with the RPIM; this difference is justified by the low density in nodes of the models. The obtained results indicate that the load is distributed along a larger cross-sectional area, which decreases the effective von Mises stress.

Figure 67 shows the obtained results by FEM for the total displacement ($|u|$) when the tube thickness on top increases.

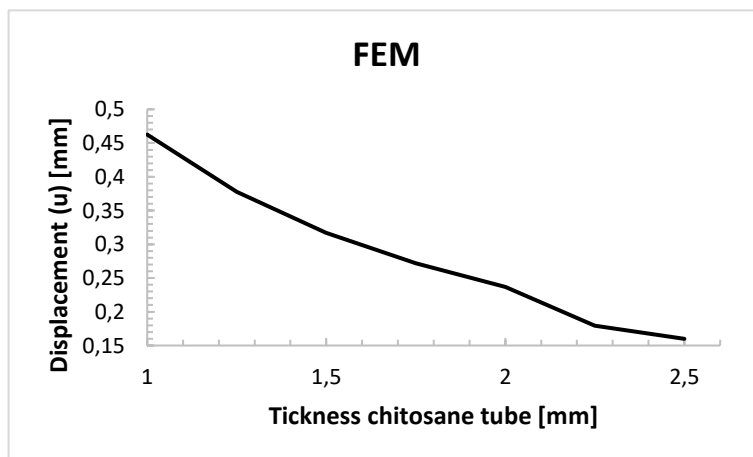


Figure 67 -FEM results for total displacement in function of the different thickness in the top of the chitosan tube.

The FEM results show that the total displacement decreases when the tube wall thickness increases; it is almost a linear decrease.

Figure 68 shows the obtained results by RPIM for the total displacement ($|u|$) when the tube thickness on top increases.

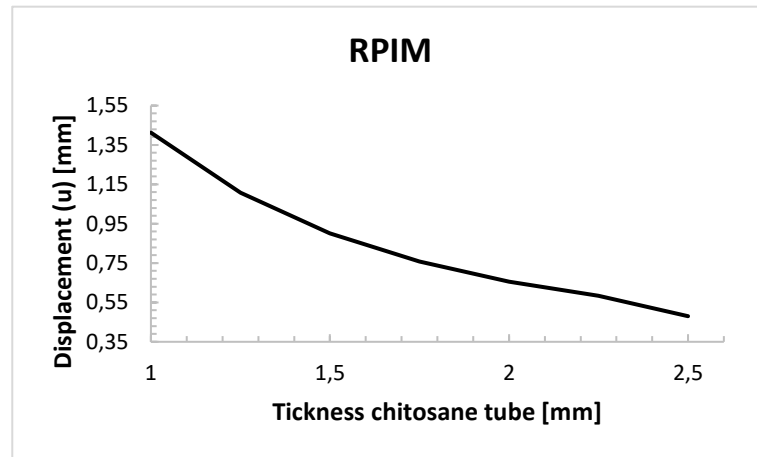


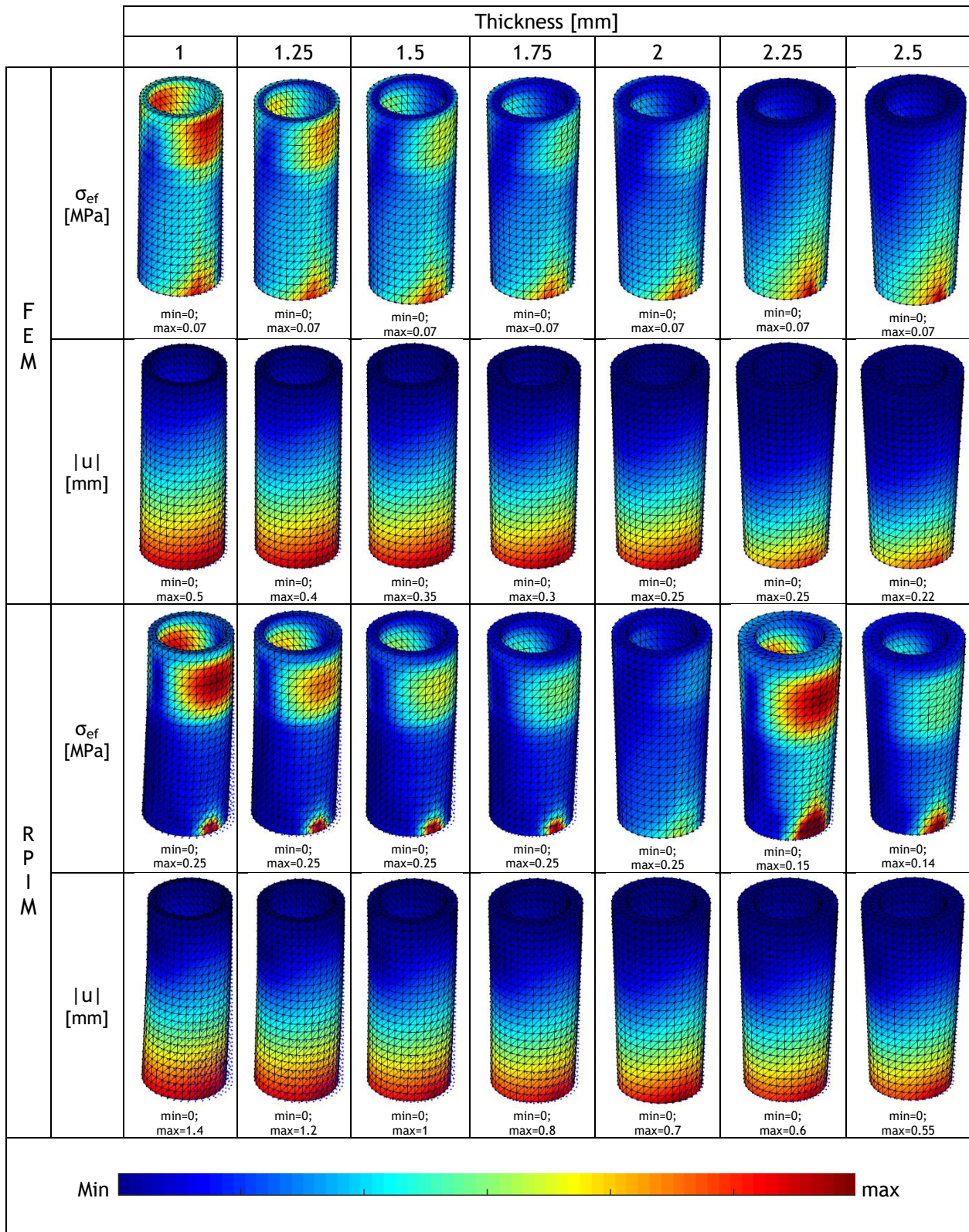
Figure 68 - RPIM results for the total displacement in function of the different thickness in the top of the chitosan tube.

When comparing the obtained results of this study with the results of the study presented in section 7.2 there are several points to mention. From a mechanical point of view the models of chitosan tubes with a different thickness in the top presents a better response when submitted to the bending test, there is a substantial decrease of the effective von Mises stress obtained in the points, corresponding to a low stress in the nerve gap which is good for a better regenerative process. The displacement also decreases when the thickness increases, comparing the usual tube, which has a fixed thickness of 1mm along the wall, with the tube with a thickness on bottom of 1mm and top of 1.5mm, the reduction of stress is higher and the difference of displacement is not significant, which means that could be a good alternative to the conventional tubes with the fix wall thickness.

The reduction of the effective von Mises stress in the nerve gap allows a better regenerative process, being this area less prone to mechanical stress, and the patient can also move in the bed during the recovering.

For better visualization of the results, the following Table 19 shows isomaps of the different results obtained with the two distinct numerical methods for all the seven different chitosan tube models, including the effective von Mises stress and total displacement ($|u|$). It is possible to see the distribution of the effective von Mises stress along the chitosan tube.

Table 19 - Isomaps of the total displacement and effective von Misesstress of the models submitted to FEM and RPIM analysis.



Chapter 8

Conclusions and Future Work

Peripheral nerve injuries have a massive impact not only on the individual subjects but also on society. The chitosan NGC's surge as a viable alternative to the conventional autologous nerve graft due to several factors, such as the reduction of surgical trauma at the repair site of the patient, the decrease of probabilities of a neuroma development and the patient does not need to sacrifice a healthy nerve [15][19]. Thus, it is imperative to study the biomechanical proprieties of chitosan tubes to improve their mechanical proprieties for better repair and regenerative process.

In my best knowledge, there are no documents regarding the study of chitosan NGC's and meshless. Therefore, in this work the use of innovative methods such as Radial Point Interpolation Method (RPIM) and the Natural Neighbour Radial Point Interpolation Method (NNRPIM) was extended for the analysis of peripheral nerve tissue in 2D and 3D models in a preliminary work and chitosan tubes in 3D models for the several elasto-static studies, being the first time that meshless methods have been used for this purpose.

The main objectives of this dissertation were to perform elasto-static analysis of chitosan tubes used for nerve regenerating using FEM and meshless methods and with these, study the effect of different parameters such as the internal and external geometry of the chitosan tube, varying the nerve suture and nerve gap length and the thickness of the tube. Another objective of this study was to compare the FEM and meshless methods performances.

At present, it is increasingly sought to propose mathematical models that seek to describe the mechanical behaviour of biomaterials and biological tissues.

In this dissertation, relevant practical conclusions were obtained. Regarding the preliminary practical works, it is possible to conclude that the performance of the three different analysis methods is very similar. Consequently, these methods are an appropriate numerical tool to analyze the biomechanical behaviour of chitosan tubes. This also confirms the robustness of the used methods. The finite element method is superior to the meshless methods in the linear process because it needs less computation capacity. However, in the nonlinear process, both FEM and meshless are equivalent regarding computational cost. Thus, meshless methods are more flexible and produce more accurate solutions, presenting an interesting potential to progress efficiently in several fields of biomechanics [20].

Frequently, in the studies founded in literature, chitosan is analyzed using a viscoelastic formulation and the nerve tissue using hyper-viscoelastic formulation, considering both isotropic materials. In this study, it was used elastic formulation to simulate chitosan. Due to the computational limitations and the computational costs that the meshless methods demand the present work not simulated big models. Nevertheless, the meshless with a reduced number of nodes can deliver accurate solutions closer to the analytic solution.

With the data obtained through the elasto-static analysis, the effect of the nerve gap length, nerve suture length and the tube wall thickness were cleared. The convergence studies demonstrate that the FEM and meshless methods are capable of giving accurate results in the structural analysis of the chitosan NGC's. However, due to the computational limitations, the models were constructed with a low node density for their dimensions. Therefore, the results obtained present a good level of accuracy.

The 3D elasto-static analysis of the internal geometry of the tube study demonstrated that the increase of the nerve gap and the nerve suture length outcomes the increase of displacement (v) of the chitosan tube.

The results represented in Figure 60 shows that the increase of the nerve gap or nerve suture length results in the decrease of effective von Mises stress felted in the chitosan tube.

The thickness of the chitosan tube wall also had a great influence in the values of the effective von Mises stress and displacement, with the increase of the wall thickness the effective von Mises stress felted in the top points of the chitosan tube reduces, and the displacement also decreases. This means that a small change in the tube thickness has a large influence on her biomechanical behaviour.

In FEM and RPIM analysis there is a marked decrease in the values of effective von Mises stress when the tube thickness of the chitosan wall is 2 mm, which means that with higher values of thickness occurs a reduction of the effective von Mises stress, subsequently inside the tube it will experience less mechanical stress for these higher values of thickness. However, the values of displacement also diminish with the increase in thickness. This decrease of displacement affects the mobility of the recovering patient because the chitosan tube becomes less flexible which means that the patient cannot perform fast movements or other wide-range movements, this also represents a less comfortable situation to the patient. Thus, the best tube solution will be the one that can resist to more significant stress and with controlled freedom of displacement. The chitosan tube with a nerve suture of 20mm and 10 mm of nerve gap will be a good choice for a reparative procedure of patient with a sciatic nerve lesion but there are other factors to take in account such has the real dimension of the nerve gap in the patient.

The study of external geometry of the tube suggest that a chitosan NGC's with a dimension of 1mm in the bottom region of the nerve suture and finishing with a thickness of 1.5 mm in the top (region of nerve gap) could be an alternative to the conventional chitosan tubes used as NGC's due to the better mechanical proprieties when compared to the tubes with a fix thickness along the wall. The tubes with a variable wall thickness present a significant reduction of the effective von Mises stress in the points A and B.

In the different studies presented in this work, the meshless methods demonstrated to be an appropriate numerical tool to analyse the biomechanical behaviour of the chitosan tubes. This also confirms the robustness and performance of the used methods when compared with the classic FEM. However, due to the power limitations, the models could not be constructed with a higher number of nodes, this limitation reduced the accuracy of the results.

For future works, it is essential to increase the nodal density of the models. New studies of chitosan tubes behaviour using these techniques should be encouraged due to the flexibility of such meshless techniques and due to the literature gap about this subject. Elastic-plastic studies should be performed in order to predict the non-linear behaviour of chitosan tubes, which will produce results closer to the real values.

References

- [1] J. Noble, C. A. Munro, V. S. S. V. Prasad, and R. Midha, "Analysis of upper and lower extremity peripheral nerve injuries in a population of patients with multiple injuries," *J. Trauma - Inj. Infect. Crit. Care*, 1998.
- [2] H. E. Rosberg, K. S. Carlsson, R. I. Cederlund, E. Ramel, and L. B. Dahlin, "Costs and outcome for serious hand and arm injuries during the first year after trauma - A prospective study," *BMC Public Health*, vol. 13, no. 1, 2013.
- [3] J. Gomes, J. Belinha, L. Dinis, and R. N. Jorge, "The structural analysis of chitosan tubes using meshless methods," *ENBENG 2017 - 5th Port. Meet. Bioeng. Proc.*, pp. 4-7, 2017.
- [4] R. S. Martins, D. Bastos, M. G. Siqueira, C. O. Heise, and M. J. Teixeira, "Traumatic injuries of peripheral nerves: a review with emphasis on surgical indication," *Arq. Neuropsiquiatr.*, vol. 71, no. 10, pp. 811-814, 2013.
- [5] E. A. Deitch and W. R. Grimes, "Experience with 112 shotgun wounds of the extremities," *J. Trauma - Inj. Infect. Crit. Care*, 1984.
- [6] S. Jones, H. M. Eisenberg, and X. Jia, "Advances and future applications of augmented peripheral nerve regeneration," *Int. J. Mol. Sci.*, vol. 17, no. 9, pp. 1-17, 2016.
- [7] P. Saxena, L. Cutler, and L. Feldberg, "Assessment of the severity of hand injuries using 'hand injury severity score', and its correlation with the functional outcome," *Injury*, vol. 35, no. 5, pp. 511-516, 2004.
- [8] C. Meyer *et al.*, "Chitosan-film enhanced chitosan nerve guides for long-distance regeneration of peripheral nerves," *Biomaterials*, vol. 76, pp. 33-51, 2016.
- [9] K. Bekelis, S. Missios, and R. J. Spinner, "Falls and peripheral nerve injuries: an age-dependent relationship," *J. Neurosurg.*, vol. 1223-9, 2015.
- [10] S. Missios, K. Bekelis, and R. J. Spinner, "Traumatic peripheral nerve injuries in children: epidemiology and socioeconomics," *J. Neurosurg. Pediatr.*, vol. 14, no. 6, pp. 688-694, 2014.
- [11] W. Daly, L. Yao, D. Zeugolis, A. Windebank, and A. Pandit, "A biomaterials approach to peripheral nerve regeneration: Bridging the peripheral nerve gap and enhancing functional recovery," *J. R. Soc. Interface*, vol. 9, no. 67, pp. 202-221, 2012.
- [12] F. Gonzalez-Perez *et al.*, "Tubulization with chitosan guides for the repair of long gap peripheral nerve injury in the rat," *Microsurgery*, vol. 300-8, 2015.
- [13] C. T. Chalfoun, G. A. Wirth, and G. R. D. Evans, "Tissue engineered nerve constructs: Where do we stand?," *J. Cell. Mol. Med.*, vol. 10, no. 2, pp. 309-317, 2006.
- [14] B. Battiston, S. Geuna, M. Ferrero, and P. Tos, "Nerve repair by means of tubulization: Literature review and personal clinical experience comparing biological and synthetic conduits for sensory nerve repair," *Microsurgery*, vol. 25, no. 4, pp. 258-

- 267, 2005.
- [15] Y. Shapira *et al.*, "Comparison of results between chitosan hollow tube and autologous nerve graft in reconstruction of peripheral nerve defect: An experimental study," *Microsurgery*, vol. 36, pp. 664-671, 2016.
 - [16] I. Y. Kim *et al.*, "Chitosan and its derivatives for tissue engineering applications," *Biotechnol. Adv.*, vol. 26, no. 1, pp. 1-21, 2008.
 - [17] L. H. Li, J. C. Deng, H. R. Deng, Z. L. Liu, and L. Xin, "Synthesis and characterization of chitosan/ZnO nanoparticle composite membranes," *Carbohydr. Res.*, vol. 345, no. 8, pp. 994-998, 2010.
 - [18] T. Kean and M. Thanou, "Biodegradation, biodistribution and toxicity of chitosan," *Adv. Drug Deliv. Rev.*, vol. 62, no. 1, pp. 3-11, 2010.
 - [19] Y. C. Huang and Y. Y. Huang, "Biomaterials and strategies for nerve regeneration," *Artif. Organs*, vol. 30, no. 7, pp. 514-522, 2006.
 - [20] J. Belinha, *Meshless Methods in Biomechanics: Bone Tissue Remodelling Analysis*. 2014.
 - [21] U. Z. Sladjana, J. D. Ivan, and S. D. Bratislav, "Microanatomical structure of the human sciatic nerve," *Surg. Radiol. Anat.*, vol. 30, no. 8, pp. 619-626, 2008.
 - [22] R. D. Keynes, D. J. Aidley, and C. L.-H. Huang, *Nerve and Muscle*. Cambridge: Cambridge University Press, 2010.
 - [23] R. Birch, *Surgical disorders of the peripheral nerves*. 2011.
 - [24] J. P. Barral, "Manual Therapy for the Peripheral Nerves," in *Manual Therapy for the Peripheral Nerves*, 2007, pp. 15-124.
 - [25] H. You, "Introduction to the FEM," 2011, pp. 278-308.
 - [26] R. S. Spinner, E. Tubbs, M. Rizk, M. Shoja, N. Loukas, and R. Barbaro, "Human Dermatomes," in *Nerves and Nerve Injuries*, 2015.
 - [27] R. Baxter, N. Hastings, A. Law, and E. J. . Glass, *A rapid and robust sequence-based genotyping method for BoLA-DRB3 alleles in large numbers of heterozygous cattle.*, vol. 39, no. 5. 2008.
 - [28] S. Peltonen, M. Alanne, and J. Peltonen, "Barriers of the peripheral nerve," *Tissue Barriers*, no. e24956, 2013.
 - [29] Y. Olsson and K. Kristensson, "The perineurium as a diffusion barrier to protein tracers following trauma to nerves," *Acta Neuropathol.*, vol. 23, no. 2, pp. 105-111, 1973.
 - [30] R. S. Tubbs, E. Rizk, M. M. Shoja, M. Loukas, N. Barbaro, and R. J. Spinner, *Nerves and Nerve Injuries*. 2015.
 - [31] W. F. Penfold, "On Injuries to Peripheral Nerves. (Pract., Aug., 1899.) Horsley, V," *J. Ment. Sci.*, vol. 46, no. 193, p. 371, 1900.
 - [32] J. Haftek, "Stretch injury of peripheral nerve. Acute effects of stretching on rabbit nerve.," *J. Bone Joint Surg. Br.*, vol. 52, no. 2, pp. 354-365, 1970.
 - [33] W. B. Highet and W. Holmes, "Traction injuries to the lateral popliteal nerve and traction injuries to peripheral nerves after suture," *Br. J. Surg.*, 1943.
 - [34] P. Fleming, B. Lenahan, S. O'Rourke, P. McHugh, K. Kaar, and J. P. McCabe, "Strain on the human sciatic nerve in vivo during movement of the hip and knee," *J. Bone Jt. Surg.*, vol. 85, no. 3, pp. 363-365, 2003.
 - [35] R. M. Menorca, T. S. Fussell, and J. C. Elfar, "Nerve physiology: mechanisms of injury and recovery.," *Hand Clin.*, 2013.
 - [36] T. Ding, Z. J. Luo, Y. Zheng, X. Y. Hu, and Z. X. Ye, "Rapid repair and regeneration of damaged rabbit sciatic nerves by tissue-engineered scaffold made from nano-silver and collagen type I," *Injury*, vol. 41, no. 5, pp. 522-527, 2010.
 - [37] K. S. Topp and B. S. Boyd, "Structure and biomechanics of peripheral nerves: nerve responses to physical stresses and implications for physical therapist practice.," vol. 86, no. 1, pp. 92-109, 2006.
 - [38] H. Millesi, G. Zoch, and R. Reihnsner, "Mechanical Properties of Peripheral Nerves," no. 314, pp. 76-83, 1995.
 - [39] J. A. Beel, D. E. Groswald, and M. W. Luttges, "Alterations in the mechanical properties of peripheral nerve following crush injury," *J. Biomech.*, 1984.
 - [40] R. A. Abrams, J. M. Butler, S. Bodine-Fowler, and M. J. Botte, "Tensile properties of the neurorrhaphy site in the rat sciatic nerve," *J. Hand Surg. Am.*, 1998.
 - [41] E. B. Toby and D. Hanesworth, "Ulnar nerve strains at the elbow," *J. Hand Surg. Am.*,

- 1998.
- [42] C. Byl, C. Puttlitz, N. Byl, J. Lotz, and K. Topp, "Strain in the median and ulnar nerves during upper-extremity positioning," *J. Hand Surg. Am.*, 2002.
 - [43] D. L. McLellan and M. Swash, "Longitudinal sliding of the median nerve during movements of the upper limb," *J. Neurol. Neurosurg. Psychiatry*, 1976.
 - [44] G. Liu, Q. Zhang, Y. Jin, and Z. Gao, "Stress and strain analysis on the anastomosis site sutured with either epineurial or perineurial sutures after simulation of sciatic nerve injury.," *Neural Regen. Res.*, 2012.
 - [45] C. T. Chang, Y. H. Chen, C. C. K. Lin, and M. S. Ju, "Finite element modeling of hyper-viscoelasticity of peripheral nerve ultrastructures," *J. Biomech.*, vol. 48, no. 10, pp. 1982-1987, 2015.
 - [46] E. Giannessi, M. R. Stornelli, and P. N. Sergi, "A unified approach to model peripheral nerves across different animal species," *PeerJ*, vol. 5, p. e4005, 2017.
 - [47] D. M. Hamby, "A comparison of sensitivity analysis techniques," *Health Phys.*, 1995.
 - [48] V. Dodane and V. D. Vilivalam, "Pharmaceutical applications of chitosan," *Pharmaceutical Science and Technology Today*. 1998.
 - [49] B. Krajewska, "Membrane-based processes performed with use of chitin/chitosan materials," in *Separation and Purification Technology*, 2005.
 - [50] M. Dash, F. Chiellini, R. M. Ottenbrite, and E. Chiellini, "Chitosan - A versatile semi-synthetic polymer in biomedical applications," *Prog. Polym. Sci.*, vol. 36, no. 8, pp. 981-1014, 2011.
 - [51] K. Kamiyama, H. Onishi, and Y. Machida, "Biodisposition characteristics of N-succinyl-chitosan and glycol-chitosan in normal and tumor-bearing mice.," *Biological & pharmaceutical bulletin*. 1999.
 - [52] K. S. Chow and E. Khor, "Novel fabrication of open-pore chitin matrixes," *Biomacromolecules*, 2000.
 - [53] S. V. Madhally and H. W. T. Matthew, "Porous chitosan scaffolds for tissue engineering," *Biomaterials*, 1999.
 - [54] F. Croisier and C. Jérôme, "Chitosan-based biomaterials for tissue engineering," *Eur. Polym. J.*, 2013.
 - [55] H. Nishikawa *et al.*, "Sulfated glycosaminoglycan synthesis and its regulation by transforming growth factor-beta in rat clonal dental pulp cells.," *J. Endod.*, 2000.
 - [56] G. Ciardelli and V. Chiono, "Materials for peripheral nerve regeneration," *Macromolecular Bioscience*. 2006.
 - [57] S. Itoh *et al.*, "Hydroxyapatite-coated tendon chitosan tubes with adsorbed laminin peptides facilitate nerve regeneration in vivo," *Brain Res.*, 2003.
 - [58] A. Matsuda, H. Kobayashi, S. Itoh, K. Kataoka, and J. Tanaka, "Immobilization of laminin peptide in molecularly aligned chitosan by covalent bonding," *Biomaterials*, 2005.
 - [59] M. E. Chavez-Delgado *et al.*, "Facial nerve regeneration through progesterone-loaded chitosan prosthesis. A preliminary report.," *J. Biomed. Mater. Res. B. Appl. Biomater.*, 2003.
 - [60] M. Cheng, K. Gong, J. Li, Y. Gong, N. Zhao, and X. Zhang, "Surface modification and characterization of chitosan film blended with poly-L-lysine," *J. Biomater. Appl.*, 2004.
 - [61] S. Dumitriu and V. Popa, *Polymeric biomaterials: Structure and function*. 2013.
 - [62] R. H. Chen and H. D. Hwa, "Effect of molecular weight of chitosan with the same degree of deacetylation on the thermal, mechanical, and permeability properties of the prepared membrane," *Carbohydr. Polym.*, 1996.
 - [63] H. R. Le, S. Qu, R. E. Mackay, and R. Rothwell, "Fabrication and mechanical properties of chitosan composite membrane containing hydroxyapatite particles," *J. Adv. Ceram.*, 2012.
 - [64] J. A. Lopes Da Silva and C. A. N. S. Santos, "Linear viscoelastic behavior of chitosan films as influenced by changes in the biopolymer structure," *J. Polym. Sci. Part B Polym. Phys.*, 2007.
 - [65] M. Ahearne, Y. Yang, A. J. El Haj, K. Y. Then, and K. K. Liu, "Characterizing the viscoelastic properties of thin hydrogel-based constructs for tissue engineering applications," *J. R. Soc. Interface*, 2005.

- [66] A. Ghanem and M. Katalinich, "Characterization of chitosan films for tissue engineering applications," *Appl. Bionics Biomech.*, vol. 2, no. 1, pp. 9-16, 2005.
- [67] K. S. Anseth, C. N. Bowman, and L. Brannon-Peppas, "Mechanical properties of hydrogels and their experimental determination," *Biomaterials*. 1996.
- [68] M. T. Shaw and W. J. MacKnight, *Introduction to Polymer Viscoelasticity: Third Edition*. 2005.
- [69] P. Magne, "Efficient 3D finite element analysis of dental restorative procedures using micro-CT data," *Dent. Mater.*, vol. 23, no. 5, pp. 539-548, 2007.
- [70] O. Zienkiewicz, R. Taylor, and J. Z. Zhu, *The Finite Element Method: its Basis and Fundamentals: Seventh Edition*. 2013.
- [71] J.-P. Geng, K. B. C. Tan, and G.-R. Liu, "Application of finite element analysis in implant dentistry: A review of the literature," *J. Prosthet. Dent.*, 2001.
- [72] C. Ko and T. D. Brown, "A fluid-immersed multi-body contact finite element formulation for median nerve stress in the carpal tunnel," *Comput. Methods Biomech. Biomed. Engin.*, vol. 10, no. 5, pp. 343-349, 2007.
- [73] A. Srirekha and K. Bashetty, "Infinite to finite: An overview of finite element analysis," *Indian J. Dent. Res.*, 2010.
- [74] W. Li, M. V. Swain, Q. Li, J. Ironside, and G. P. Steven, "Fibre reinforced composite dental bridge. Part II: Numerical investigation," *Biomaterials*, 2004.
- [75] C. Tavares, J. Belinha, L. Dinis, and R. N. Jorge, "The numerical analysis of a restored tooth using meshless methods," *Proc. - 2015 IEEE 4th Port. Meet. Bioeng. ENBENG 2015*, 2015.
- [76] C. S. S. Tavares, J. Belinha, L. M. J. S. Dinis, and R. M. Natal Jorge, "The elasto-plastic response of the bone tissue due to the insertion of dental implants," in *Procedia Engineering*, 2015.
- [77] W. K. Liu, S. Jun, and Y. F. Zhang, "Reproducing kernel particle methods," *Int. J. Numer. Methods Fluids*, 1995.
- [78] B. Nayroles, G. Touzot, and P. Villon, "Generalizing the finite element method: Diffuse approximation and diffuse elements," *Comput. Mech.*, 1992.
- [79] T. Belytschko, Y. Y. Lu, and L. Gu, "Element-free Galerkin methods," *Int. J. Numer. Methods Eng.*, 1994.
- [80] L. M. J. S. Dinis, R. M. Natal Jorge, and J. Belinha, "Analysis of 3D solids using the natural neighbour radial point interpolation method," *Comput. Methods Appl. Mech. Eng.*, 2007.
- [81] J. Belinha, L. M. J. S. Dinis, and R. M. N. Jorge, "The analysis of the bone remodelling around femoral stems: A meshless approach," *Math. Comput. Simul.*, 2015.
- [82] R. L. Hardy, "Multiquadric equations of topography and other irregular surfaces," *J. Geophys. Res.*, 1971.
- [83] J. G. Wang and G. R. Liu, "A point interpolation meshless method based on radial basis functions," *Int. J. Numer. Methods Eng.*, 2002.
- [84] O. C. Zienkiewicz, R. L. Taylor, and R. L. Taylor, *The Finite Element Method for Solid and Structural Mechanics*. 2005.
- [85] G. R. Liu and S. S. Quek, *Finite Element Method: A Practical Course*. 2003.
- [86] H. Street, "Finite Element Analysis on Limb," 1983.
- [87] D. Morata, *Finite Element Analysis - From Biomedical Applications to Industrial Developments*. 2018.
- [88] C. T. Chang, Y. H. Chen, C. C. K. Lin, and M. S. Ju, "Finite element modeling of hyper-viscoelasticity of peripheral nerve ultrastructures," *J. Biomech.*, 2015.
- [89] C. Ko and T. D. Brown, "A fluid-immersed multi-body contact finite element formulation for median nerve stress in the carpal tunnel," *Comput. Methods Biomech. Biomed. Engin.*, 2007.
- [90] K. Rahmani-Monfard, A. Fathi, and S. M. Rabiee, "Three-dimensional laser drilling of polymethyl methacrylate (PMMA) scaffold used for bone regeneration," *Int. J. Adv. Manuf. Technol.*, 2016.
- [91] A. Sasson, S. Patchornik, R. Eliasy, D. Robinson, and R. Haj-Ali, "Hyperelastic mechanical behavior of chitosan hydrogels for nucleus pulposus replacement- Experimental testing and constitutive modeling," *J. Mech. Behav. Biomed. Mater.*, 2012.

- [92] S. Elangovan and G. M. Odegard, "Finite Element Modeling of Intraneural Ganglion Cysts of the Common Peroneal Nerve," *Vol. 11 Mech. Solids, Struct. Fluids*, no. January 2009, pp. 103-105, 2009.
- [93] J. T. Maikos, Z. Qian, D. Metaxas, and D. I. Shreiber, "Finite Element Analysis of Spinal Cord Injury in the Rat," *J. Neurotrauma*, 2008.
- [94] X. Wang, A. J. Neely, G. G. McIlwaine, M. Tahtali, T. P. Lillicrap, and C. J. Lueck, "Finite element modeling of optic chiasmal compression," *J. Neuro-Ophthalmology*, vol. 34, no. 4, pp. 324-330, 2014.
- [95] A. Shin, L. Yoo, J. Park, and J. L. Demer, "Finite Element Biomechanics of Optic Nerve Sheath Traction in Adduction," *J. Biomech. Eng.*, vol. 139, no. 10, p. 101010, 2017.
- [96] J. R. Andrade, J. Belinha, L. M. J. S. Dinis, and R. M. N. Jorge, "Analysis of dental implant using a meshless method," *Biodental Eng. II - Proc. 2nd Int. Conf. Biodental Eng. BIODENTAL 2012*, pp. 145-150, 2014.
- [97] A. Jose, "Estudo Comparativo De Tensões Aplicadas À Cabeça Do Femur Em Uma Prótese De Quadril Através De Sistema Computacional," vol. 12, no. 22, 2011.
- [98] M. S. Ju, C. C. K. Lin, and C. T. Chang, "Researches on biomechanical properties and models of peripheral nerves - A review," *J. Biomech. Sci. Eng.*, vol. 12, no. 1, 2017.
- [99] "REAXON ® NERVE GUIDE - NERVE CONDUIT MADE OF CHITOSAN (accessed January 2019)." [Online]. Available: <https://www.kerimedical.com/en/portail-kerimedical-en/home-professional/reaxon-product-en/>.