

Characterization of the Mandible *Atta Laevigata* and the Bioinspiration for the Development of a Biomimetic Surgical Clamp

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Received: December 30, 2016; Revised: June 19, 2017; Accepted: July 21, 2017

Approximately thousand years ago it was reported the use of mandibles of ants for suture. In this sense, bioinspired components, as absorbable surgical clamps, can be designed. This study is aimed to characterize the mandible of the ant *Atta laevigata* in order to help the selection of candidate biomaterials for application as surgical clamps. Three pairs of mandibles were used and ten nanoindentations were performed in each pair. The average hardness for the samples in the internal and external regions were 0.36 ± 0.06 GPa and 0.19 ± 0.04 GPa, respectively and the average elastic modulus for the internal and external regions were 6.16 ± 0.23 GPa and 2.74 ± 0.44 GPa, respectively. The morphology of the mandible was observed in detail by scanning electron microscopy, as well as Energy-dispersive X-ray spectroscopy. The average roughnesses on the internal and external regions, measured by atomic force microscopy, were 6.73 ± 0.90 nm and 11.87 ± 1.42 nm, respectively. From these results, it was possible to identify biomaterials that mimic the mandible behaviour for surgical clamp.

Keywords: *Ant Atta laevigata, Characterization, Surgical clamp and Biomaterials.*

1. Introduction

Since antiquity, a great number of materials have been tested and used for suture of injuries, such as vegetable fibers, tendons, intestines of many animals, horse mane, golden filaments, among others¹. There are registers of the use of sutures with linen and gold in Ancient Egypt, as well as the use of cat's intestine in Europe, during the Middle Age². Approximately thousand years ago, the use of mandible of ants for the approximation of the edges of an injury has been reported in the medical Indian text Charaka Sanhita³. Based on this knowledge, an absorbable surgical clamp (MU9102934-1) has been designed, emulating the mechanics of bite produced by the mandible of the ant *Atta laevigata*. This clamp proposed aims to simplify the suture for both placing and the removing from the skin, in a less traumatic and efficient condition for the patient. The suture clamp is composed by the handles and approximation systems⁴. The following step is based on the materials selection of candidates to be used in the surgical clamps, keeping in mind that such materials must be biocompatible. For the handles system, the material must present high elasticity, so to be able to join the edges of the wound for the scarring and, for the approximation system of the clamp the material must be mechanically resistant and bio-absorbable. In order to develop the surgical clamp similar to the mandible, it is necessary

to select materials based on a detailed characterization of the properties of the ant mandible. Therefore, in this work, mechanical nanoindentation, scanning electron microscope (SEM), energy-dispersive X-ray spectroscopy (EDS) and Atomic Force Microscopy (AFM) were used for the investigation of the mandible, in order to identify and correlate its properties with the possible biomaterials that can be applied in the bioinspired surgical clamp.

2. Materials

2.1 Biological materials

Biological materials have been widely used in the past and can still applied or emulated today in Medicine. The properties of biological materials drive scientists and engineers to create new materials or to improve the existing ones. In this regard, it is very important to understand the properties of the biological materials as well as the understanding of the zoology and of the behavior of the living systems^{5,6}.

The structures and the characteristics of biological materials can be distinguished among them, and their mechanical properties can be correlated with the synthetic materials according to Table 1⁶⁻¹⁵.

The biological material of mandibles of insects can be usually correlated with polymers since; in general, they are

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Table 1. Elastic modulus and tensile strength of biological and synthetic materials¹⁵.

Synthetic materials/ Biological Materials	E (MPa)	σ (MPa)	Reference
1. Biopolymer			
Cellulose	150.000	18	7
Collagen (along fibers)	1000	50-100	8,7
Fibroin	10.000	70	7
Keratin (wool)	5000	200	9
Cuticle (insect)	0.001-20.000	60-200	10,11
2. Biominerals			
Hydroxyapatite (from enamel)	83.000	60	12-14
Zirconia	200.000	250	6
Silicon Carbide (SiC)	450.000	3500	6
3. Biocomposites			
Trabecular bone	800-14.000	1-100	15
Cortical bone	6000-20.000	30-150	15

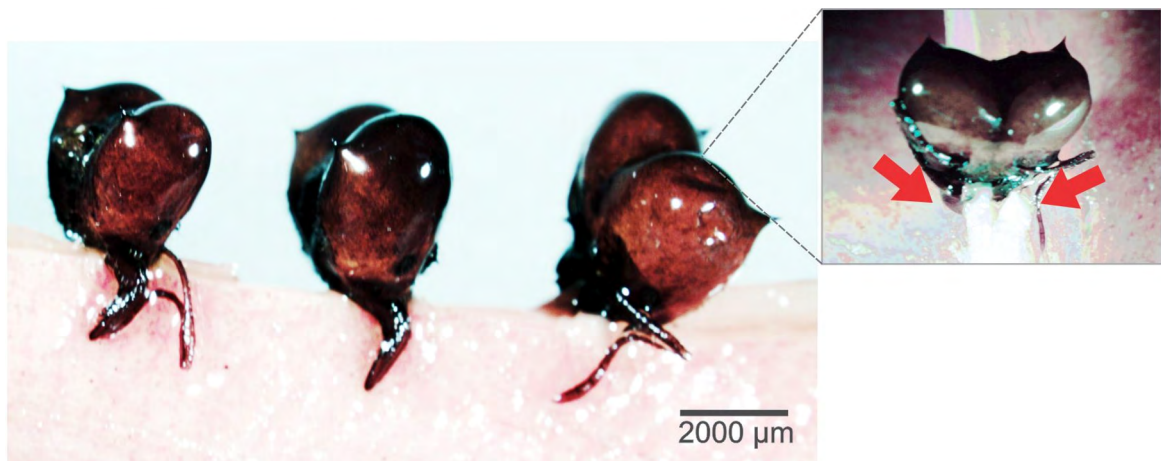
composed by waxes, polysaccharides and proteins. Furthermore, as comparison, jaws of animals are twice more rigid than the exoskeleton due to the presence of metals, like zinc, manganese, iron and, in some cases, calcium. The element zinc is the main responsible for the increase of 20% of the hardness of the natural material¹⁶⁻²¹. The use of mandibles of the ant *Atta laevigata* in the suture is efficient, because presents form and mechanics able to join the edges of the wound⁴, as well as it is made of a naturally resistant material. This use of mandible of the ant *Atta laevigata* acting as a suture component is illustrated in Figure 1.

The species *Atta laevigata* presents a large head, composed by muscular fibers annexed through the apodeme and interconnected with the mandible, allowing strong mandible movements^{22,23}. For the functioning of the suture clamp by the mechanism inspired by the ant's mandible, an external compressive stress must be applied to the levers to force its opening and, when open, the approximation system of the surgical clamp is able to penetrate the skin based on elastic forces. Another important characteristic of the approximation

system of the proposed surgical clamp is to be able to fall by itself after the healing⁴. Figure 2 shows a scheme of the functioning of the surgical clamp in the suture analogue to the functioning of the natural system.

2.2 Biomaterials

The materials used for medical applications can be divided in four categories: metals, polymers, ceramics and composites²⁴. However, metals and polymers are the natural candidates of biomaterials for due to their mechanical, chemical and surface properties. Metals are already largely employed for substitution, reinforcement or stabilization of rigid tissues. They present improved mechanical performance, high mechanical and fracture resistance, durability and possibility of surface polishing and abrasion²⁵⁻²⁸. The main groups of biomaterials are the stainless steels, the titanium alloys, commercially pure titanium and cobalt-chrome based alloys^{25,29}. Polymers are less demanding regarding the manufacture of varied forms and a relatively large availability of different classes with mechanical and physical

**Figure 1.** Suture with the mandible of the ant *Atta laevigata*.

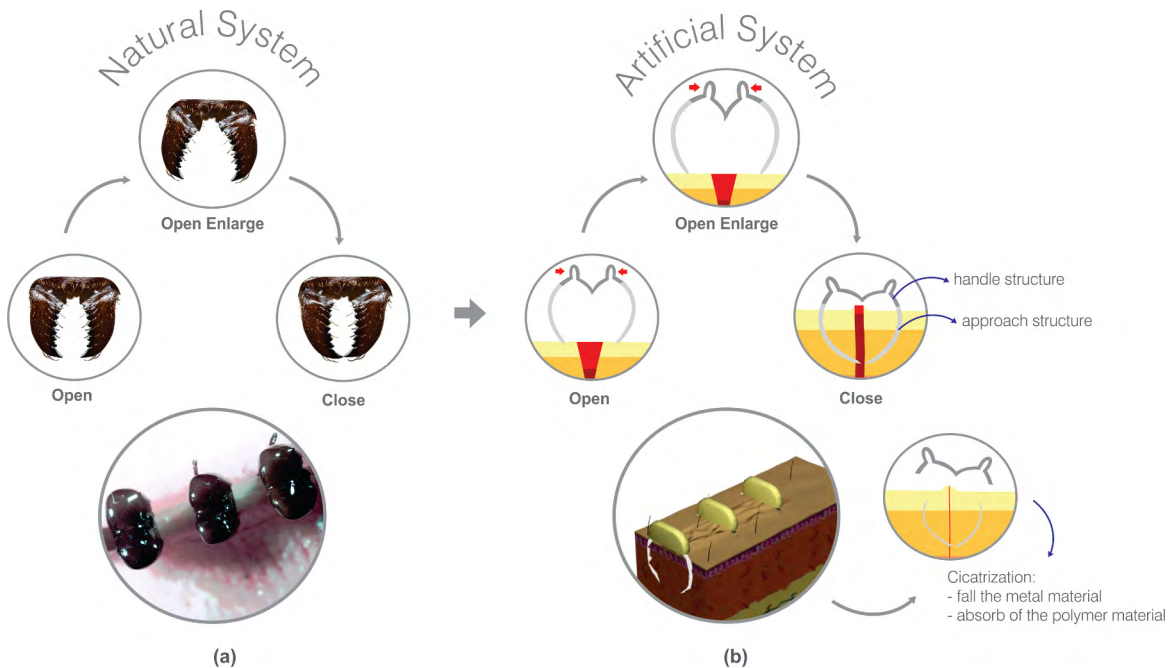


Figure 2. Mechanism of bite of the ant's mandible *Atta laevigata* in the suture (a) and functioning of the biomimetic surgical clamp in the suture (b).

properties for specific applications³⁰. The main types of synthetic polymers are polylactic acid (PLA), polyglycolic acid (PGA) and the copolymer poly(lactic-co-glycolic acid) (PLGA)³¹. These polymers have been used in biodegradable sutures, absorbable devices for bone fixation and sources for controllable drug release³². As more frequently used natural polymers are proteins (collagen, elastin and silk fibroin) and polysaccharides (chitosan, alginate, hyaluronic acid and pectin)^{33,34}. The main applications of these biopolymers are in wound treatments and controllable drug release^{35,36}. It is worth mentioning that the combination between two or more synthetic or natural polymers in the form of complexes or blends, makes it possible to obtain devices with improved chemical, mechanical and biological properties when compared to the isolated polymers^{33,37,38}. In general, about the mechanical properties of the different types of biomaterials, it can be noted that the modulus of elasticity of the polymers is generally around 5 GPa, while for fibers it can reach 15 GPa, for ceramic and metallic materials, the values are approximately 9.0 to 980 GPa³⁹.

3. Materials and Methods

In this research, three samples of the mandible *Atta laevigata* were used, with approximately 0.5 mm in length. Each pair of mandible was sectioned from the ant's head, dried and embedded in epoxy resin, and polished with alcohol and alumina. Instrumented nanoindentation⁴⁰⁻⁴² was performed on the surfaces of the samples using an

Agilent G200 nanoindenter, with maximum load of 20 mN. Ten indentations were carried out on each of the samples. Scanning Electron Microscopy (SEM - JOEL, JSM - 6460 LV) was used to verify the morphology of the sample. The mandibles were covered with a gold thin film. Through Energy-dispersive X-ray spectroscopy (EDS), the metallic elements of the material were identified. For the examination of the morphological and topographic characteristics on the surface of the mandible, the atomic force microscope (AFM, 1M Plus, JPK Instruments) with non-contact tip, (type NCST-50 and dimensions 27 x 150 x 2.8 μm) were used. The arithmetic roughness (Ra)⁴³⁻⁴⁵ of the sample was determined using the AFM software through a line profile, averaging three profiles for each image of the internal and external regions of the mandible.

4. Results and Discussion

The regions in which the indentations were performed on the samples can be identified on the images obtained via optical microscopy coupled to the G200 nanoindenter, as show in Figure 3.

Ten indentations were performed on the inner surface regions, near the denticles, and on the surface of external regions of each sample, in order to verify possible differences in mechanical properties. The results of the nanoindentation on the samples are shown in Figure 4, as load displacement versus displacement into the surface plots. The displacement into surface varies a little in some samples due to irregularities in the surface.

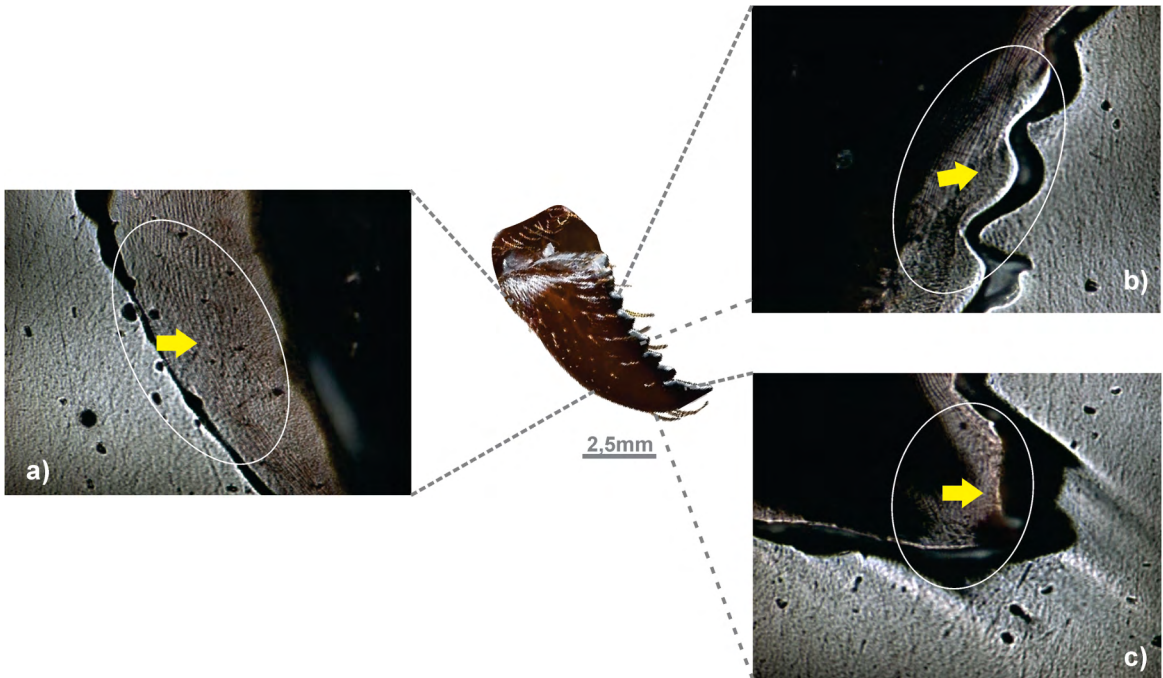


Figure 3. Nanoindentation on the regions of samples: (a) external region, (b, c) internal region of the mandible of the ant *Atta laevigata*, 10x magnification.

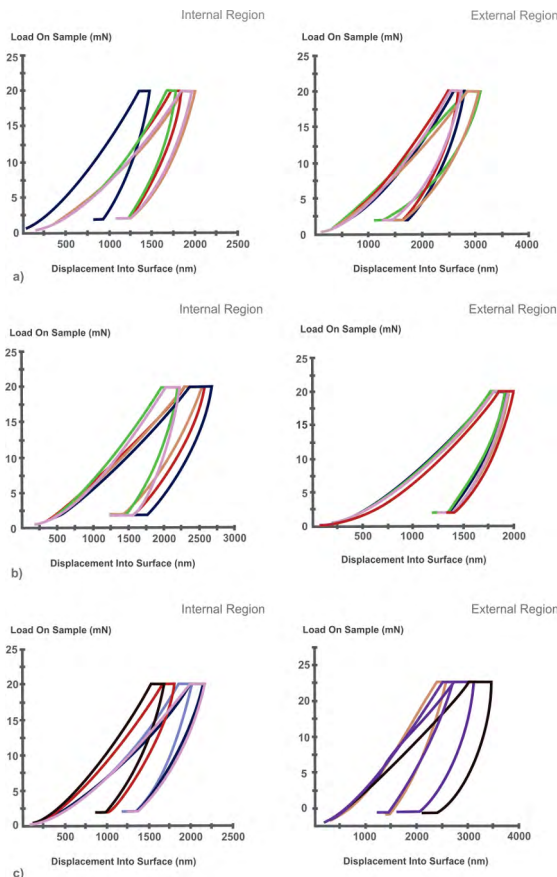


Figure 4. Load versus displacement into the surface plots on the inner and external surfaces of mandible 1 (a), mandible 2 (b) and mandible 3 (c).

Table 2 shows the comparison of nanoindentation results in the inner and external regions of mandibles 1, 2 and 3.

According to Table 2, the hardness and elastic modulus for all samples are higher in the internal region, since this region undergoes higher wear due to the strong movements that the mandible performs to cut different types of materials, indicating greater resistance compared to the external region. The average hardness of the samples in the internal and external regions was 0.36 ± 0.06 GPa and 0.19 ± 0.04 GPa, respectively. The average elastic modulus in the internal region was 6.16 ± 0.23 GPa and external of that was 2.74 ± 0.44 GPa. From these values, it is noteworthy that the material of the ant's mandible presents similarities with some groups of the polymeric materials. Among the absorbable biopolymers for application in the surgical clamp approach system, possible candidates are poly (lactic acid) (PLA), poly (glycolic acid) (PGA) and poly (ϵ -caprolactone) (PCL), with respective elastic modulus of 0.3 to 3.5 GPa, 6 to 7 GPa and 0.21 to 0.44 GPa⁴⁶, respectively. PLA and PCL are important candidates because they have been increasingly used in the medical field as they present advantages such as mechanical resistance, ability to combine with other polymer to improve physical-chemical, mechanical and biological properties, as well as non-toxicity, biocompatibility and biodegradability^{28,47}. It is also worth to mention that the natural biopolymer fibroin has been used as surgical suture because of its high mechanical resistance and biocompatibility, as well as high resistance to microorganisms^{48,49}. Another option of natural biopolymer is chitosan, as it is able to accelerate the healing

Table 2. Mechanical properties of the samples obtained by nanoindentation.

	MAND 1		MAND 2		MAND 3	
	Hardness(GPa)	Young's Modulus(GPa)	Hardness(GPa)	Young's Modulus(GPa)	Hardness(GPa)	Young's Modulus(GPa)
Internal Region	0.45 ± 0.13	6.00 ± 1.39	0.32 ± 0.01	6.41 ± 1.17	0.31 ± 0.09	5.09 ± 0.93
External Region	0.17 ± 0.02	2.28 ± 0.47	0.22 ± 0.03	3.54 ± 1.17	0.18 ± 0.09	2.40 ± 0.37

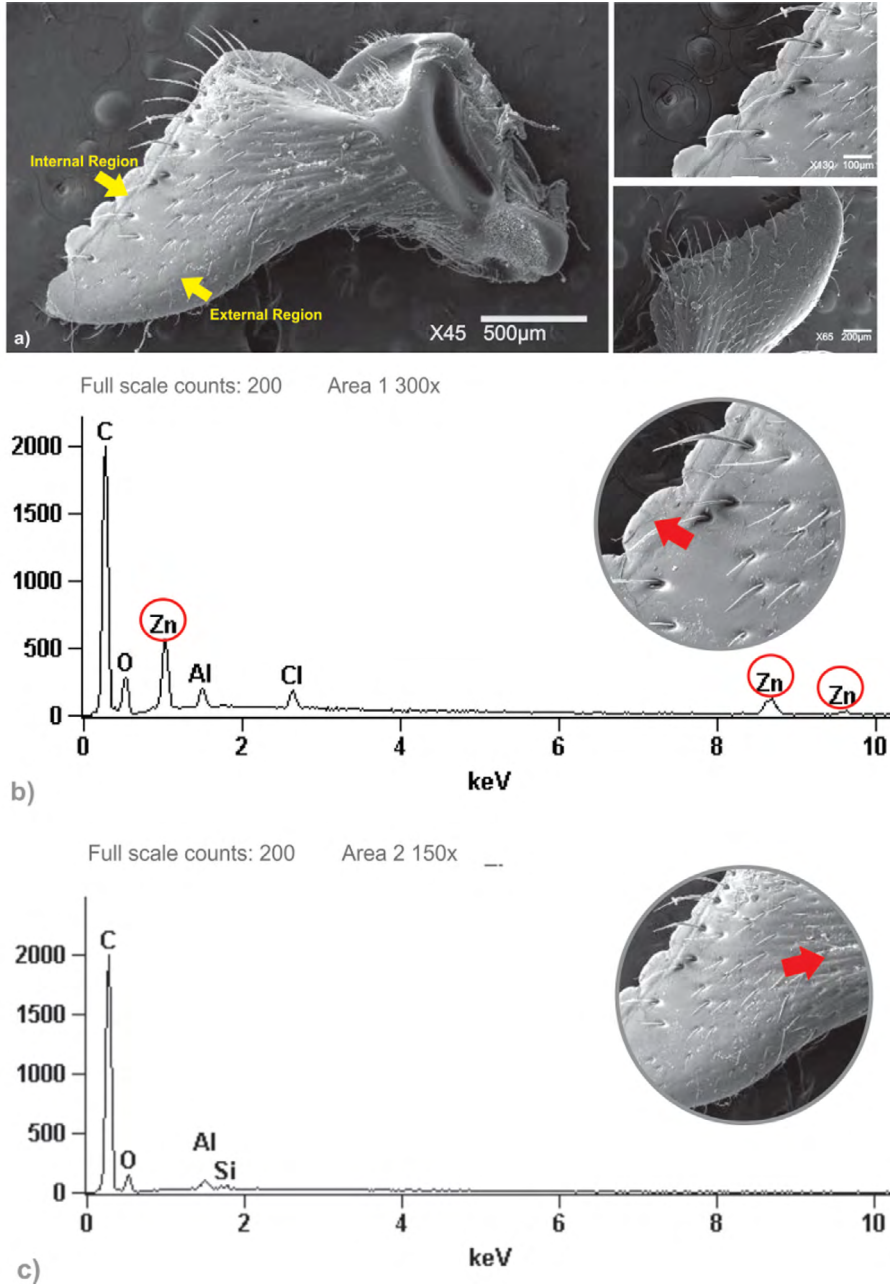


Figure 5. (a) SEM of the mandible *Atta laevigata* identifying morphological details of the internal and external regions; (b) representative analysis via EDS on the surface of the mandible, identifying metallic elements such as zinc in the internal region, (c) absence of Zn in the external area.

of lesions and the synthesis of collagen by the fibroblasts in the initial phase of healing. The combination of two more synthetic or natural polymers forming blends or polymer complexes would be an alternative to the clamp approach system, as it would increase the mechanical strength of the material, facilitating penetration of the surgical clamp approach system into the skin. This happens with the chitosan biopolymer which is combined with other polymeric materials to increase its mechanical strength^{50,51}. Another example is the combination of the natural biopolymer, like collagen with the synthetic biopolymer PCL, the objective is also to increase the mechanical properties, as this synthetic polymer exhibits high mechanical resistance⁵². Figure 5 (a) shows the morphology of the mandible of the ant *Atta laevigata* obtained by SEM, illustrating the basic external morphology

of an ant mandible: the external margin; parts of the internal margin including the masticatory margin; basal margin; basal angle; and teeth which has smooth surface. Note the groove extending on the upper right side; similar grooves and pits occur on the mandibles of many ants. Note also the row of setae (hair like structure) along the masticatory border, another common feature of ant mandibles¹⁶⁻²¹.

In addition, EDS analysis in Figure 5 (b) confirmed the presence of metals such as zinc, manganese, aluminum and halogen (chlorine) in ant's mandible samples, principally in the internal region. The analysis of the mandible by EDS indicates the presence of zinc with mass fraction of circa 6.11%. In another region of the mandible, in addition to zinc, 0.46% of manganese was also identified¹⁶⁻²¹. However, figure 5 (c) shows the absence of zinc in the external region,

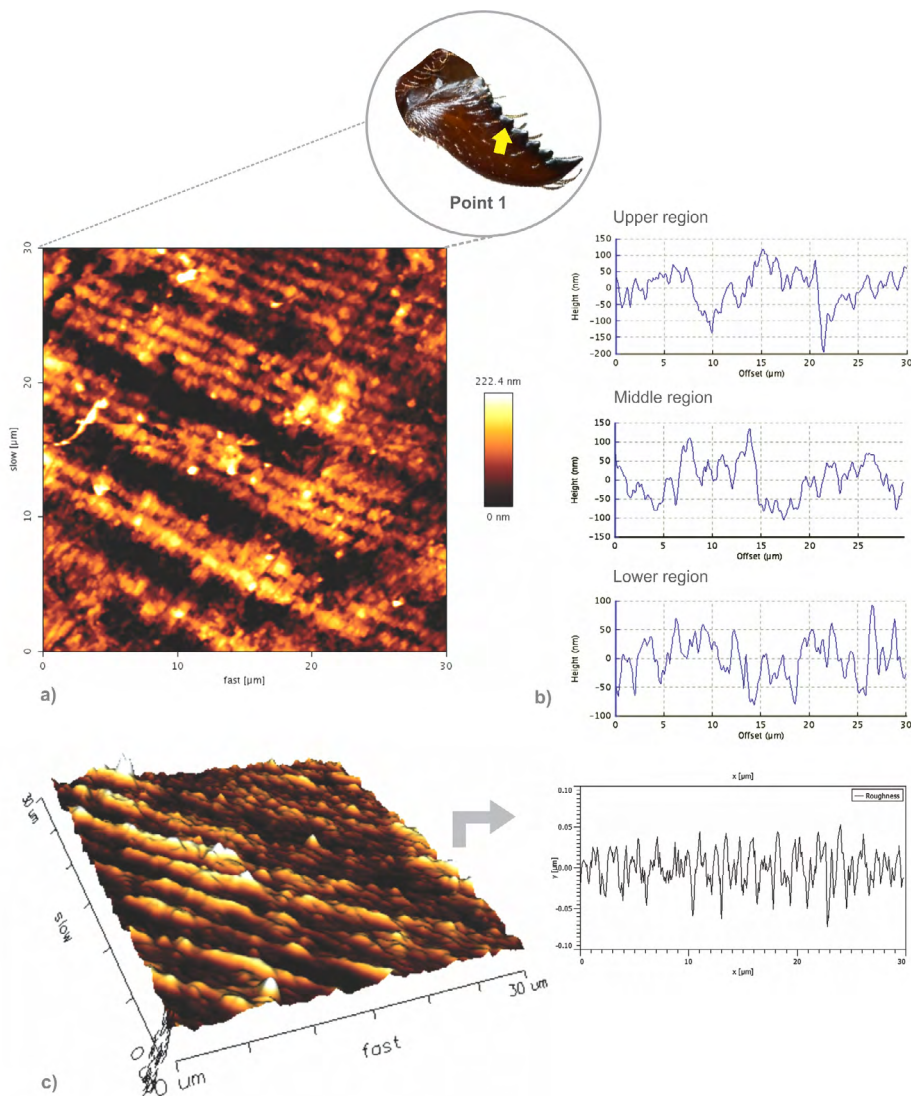


Figure 6. Topography of the internal region on the surface of the mandible of the ant *Atta laevigata* at different points, 30µm x 30µm: surface morphology (a), topographic profile in upper, middle and lower regions (b) and 3D topography (c).

so these metallic elements may influence the mechanical properties of the mandible such that the higher values of hardness and elastic modulus in the internal region than in the external one can be correlated with the occurrence of zinc and its co-located halogen chlorine., Schofield et al¹⁹ found that Zn is incorporated into the mandibular teeth of leaf-cutter ants during early adult life, they show that the hardness of the mandibular teeth increases nearly three-fold as the adults age and that hardness correlates with Zn content.

The AFM micrographs on the surface of *Atta laevigata*'s mandible are shown in Figures 6 and 7.

The AFM images present topographic similarities in both, the external and internal regions, with a certain vertical inclination of the geometry towards the same direction. The results of roughness parameter R_a obtained of the internal

and external regions of the natural material were 6.73 ± 0.90 nm and 11.87 ± 1.42 nm, respectively. These results indicate that in the mandible, the external region presents a higher roughness than the internal region. The morphology (Figura 5 (a)) of both regions can explain the topography or surface roughness. The internal region of the mandible near the denticles is much smoother than the external one which shows irregularities and presence of grooves, pits and rows of setae. Even considering all the activities performed by the mandible, the internal region demonstrated lower roughness (less wear) this may be due to the presence of zinc which enhances its mechanical strength compared to the external region. In biomaterials, surface modification, such as roughness, has been proposed to increase the surface area, adhesion of the interface between the biomaterial and

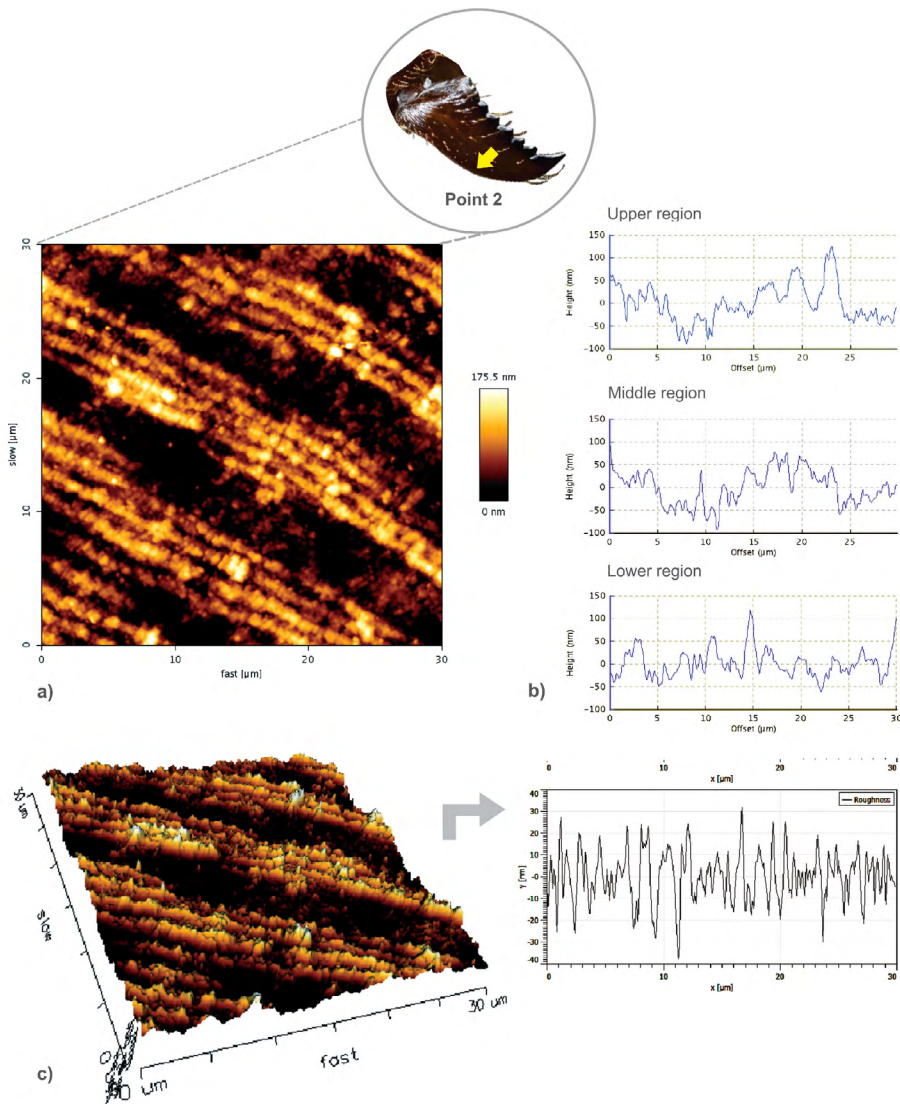


Figure 7. Topography of the external region on the surface of the mandible *Atta laevigata*, $30\mu\text{m} \times 30\mu\text{m}$: surface morphology (a), topographic profile in upper, middle and lower regions (b) and 3D topography (c).

the host⁵³. Thus, the investigation of R_a in the mandible will be useful as a parameter for the treatment of surfaces of the biomaterials selected for the surgical clamp, in order to verify the mechanical strength of the biomaterial with roughness similar to the mandible and still the adhesion of drugs to this surface.

5. Conclusion

The nanoindentation measurements showed higher hardness and elastic modulus in the internal region of the mandible of the ant *Atta laevigata*, than the external regions correlated with the presence and absence of zinc, respectively. In addition, metallic elements were found in the internal region of the mandible, such as zinc and manganese justifying the increase of hardness in this region. Morphology and topography details in the internal region, shows smooth denticles, and rougher external region of the mandible. The roughness may serve as a parameter for the surface treatment of the selected polymeric biomaterial to the bioabsorbable surgical clamp. From these results, it was possible to identify biomaterials with potential for application in the surgical clamp approach system, such as PLA, PCL or PGA, individually or in combination with natural polymers, such as chitin, collagen or fibroin, to form blends or polymer complexes. For the clamp handle system, a possible alternative is the use of metallic biomaterials such as stainless steels and titanium alloys.

6. Acknowledgements

The authors would like to acknowledge the Foundation for Research Support of the State of Amazonas (FAPEAM), National Council for Scientific and Technological Development (CNPq) for the financial support, and the Laboratory of Characterization of Surfaces of the Federal University of Rio de Janeiro (UFRJ).

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