Composition, Structural and Material Properties of Leech Teeth Example of Bioinspiration in Materials Research

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Introduction

In ancient India and Greece, leeches have been used in medicine to remove blood from patients. Today, leeching is used rarely and the use of leeches has shifted into reconstructive and plastic surgery. Although there is a number of papers dealing with the leech stretch receptors, body wall muscles [1] or central nerval system [2], there is no paper on the composition nor material properties of its teeth.

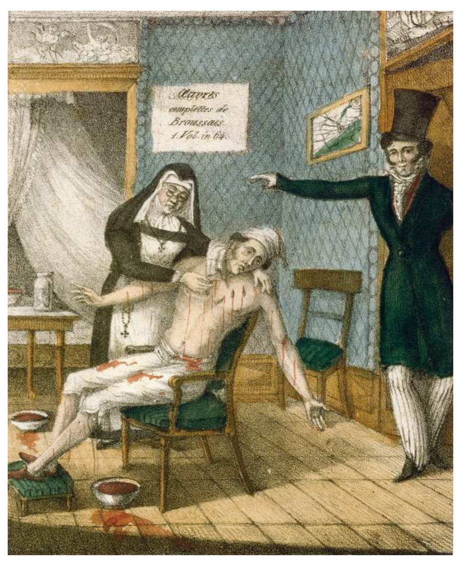

Figure: Lithograph showing the leeching of a patient [3]

The leech

The leech's sucking apparatus is an amazing instrument - it has 3 jaws and 300 teeth made for easily cut into the skin of the host animal. In this study we used nanoindentation and atomic spectroscopy to reveal composition and material properties of leeches teeth and to demonstrate the optimization possibilities of nature to manufacture these very sharp and tiny blades which can easily penetrate the host's skin.

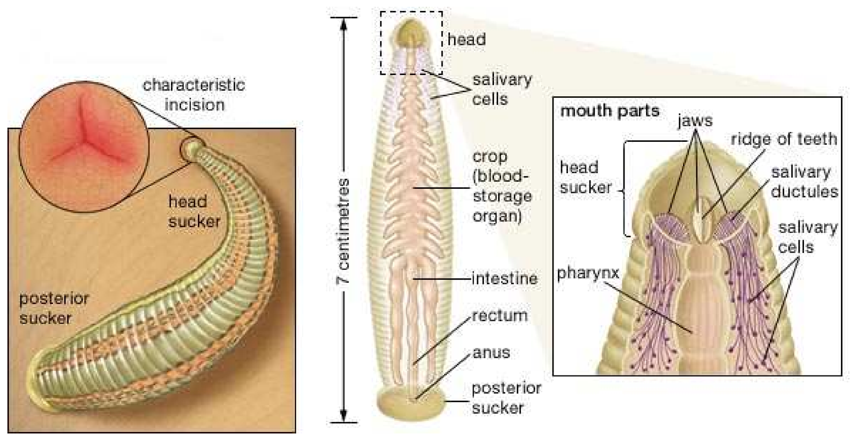

Figure: After attaching its head sucker to the skin, the leech uses its three jaws with razor-sharp teeth to make a neat Y-shaped cut. Salivary ductules between the teeth secrete several pharmacologically active substances, including a local anesthetic and the potent anticoagulant hirudin [3].

Sample extraction and preparation

Samples of leech's jaws were obtained from adult subjects of *Hemopis sanguisuga*. The subject were euthanised with ether and sliced in area of sucking apparatus. Individual jaws with length around $500\,\mu m$ were carefully separated under magnification glass ($5\times$) using a sharp-tip scalpel, microretractores and pair of tweezers. The samples were cleaned from the soft tissues and embedded in low shrinkage epoxy resin.

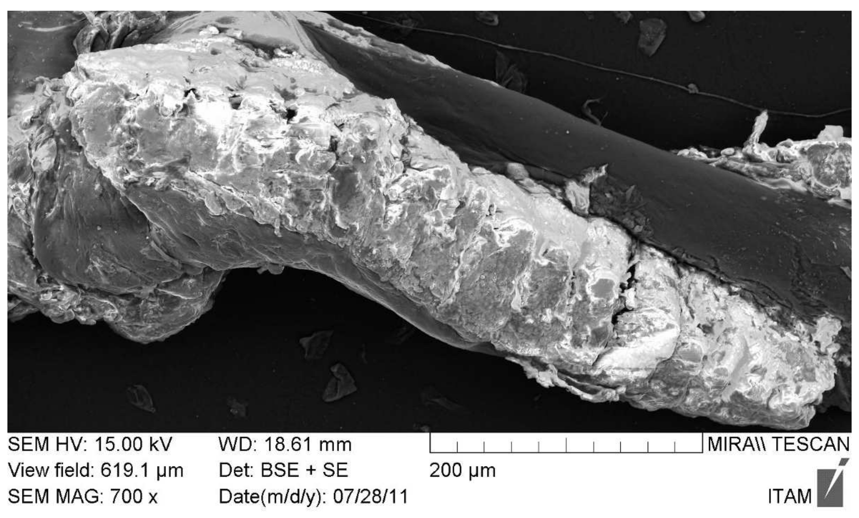

Figure: Top view on extracted leech's jaw with teeth line obtained by SEM with $700\times$ magnification

The surface of the samples was grinded and polished. Diamond grinding discs with grain size 35 and $15\,\mu m$ followed by monocrystalline diamond suspension with grain size 9, 3 and $1\,\mu m$ were used for grinding procedure [4]. The best reached final surface roughness was $R_a = 16\,nm$. All samples were prepared with roughness smaller than $R_a = 40\,nm$, which is adequate value for micromechanical testing. The roughness was measured by scanning probe microscopy (SPM).

Composition analysis

Morphological investigation of leech's teeth depicting its true size and shape has been accompanied with composition microanalysis. The microanalysis was carried out by Bruker Quantax energy dispersive spectrometer (EDS) installed in Tescan MIRA II scanning electron microscope. Concentration of individual elements was determined from the relative intensity of their characteristic X-ray spectra by the Esprit program provided by microanalyzer manufacturer. By the nature of elemental microanalysis it is impossible to identify molecular composition of the studied matter, only elemental composition is as the result available. From the composition analysis as the significant elements and their respective concentrations are calcium, oxygen and carbon were identified.

Mechanical testing

Quasi-static nanoindentation was performed using the nanomechanical instrument Hysitron TI 950 TriboIndenter^{TM}. Berkovich diamond tip, which has the shape of triangular pyramids with angle of 142.3° was used to obtain elastic properties of the teeth. The test was performed in three segments. Loading, constant force, unloading phase of the test were prescribed. Maximum force was reached at 5 second, then 2 second of dwell followed by 5 second of unloading.

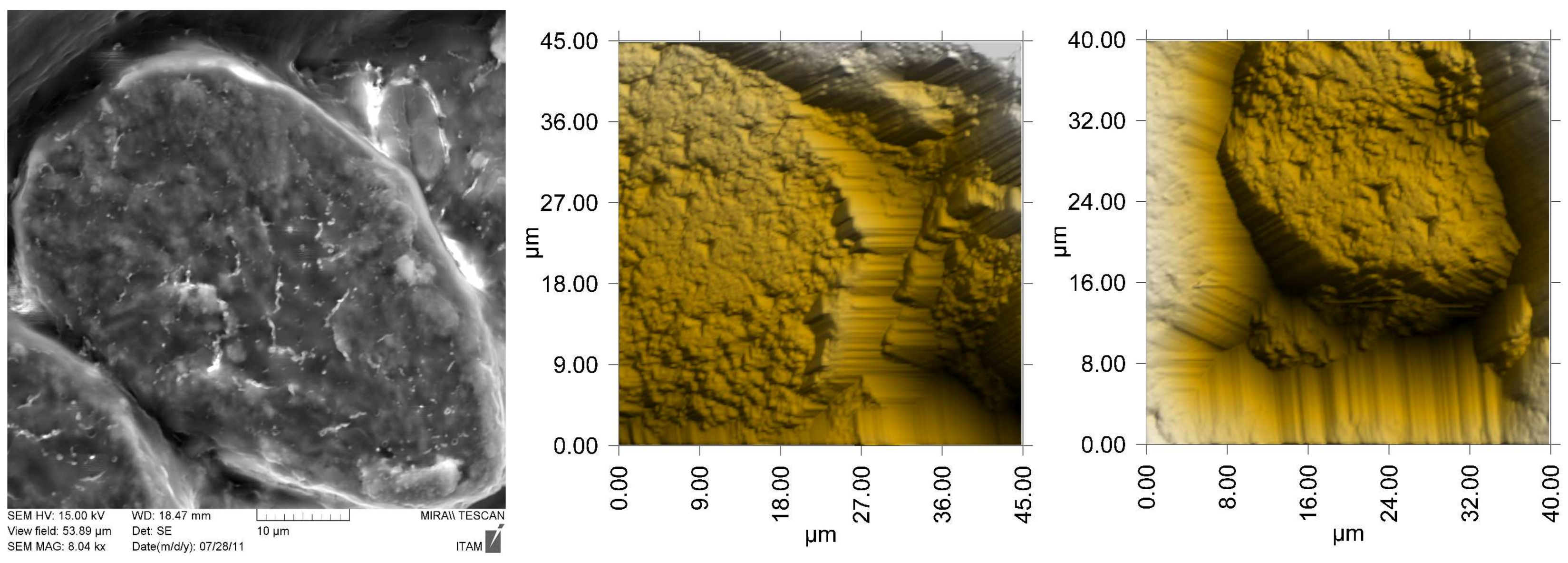

Figure: Detail of tooth obtained by SEM with $8000\times$ magnification *(left)*, Surface 3D reconstruction obtained by SPM *(right)*

The sample with minimal roughness was loaded by force $P_{max} = 900\,\mu N$ corresponding to $h_{max} \approx 150\,nm$ indents depth. To reduce of roughness effect the other samples were loaded by force $P_{max} = 8000 - 8300\,\mu N$ resulting in indents deep $h_{max} \approx 500\,nm$. All samples were indented by set of fifteen indents. For each indent force–depth curves were plotted and reduced moduli were calculated using the Oliver-Pharr method [5].

Results

In this preliminary analysis, the main constituents of leeches' teeth have been identified. The tooth is composed mainly of calcium ($41.9\,\%$), oxygen ($41.2\,\%$) and carbon ($11.4\,\%$), other constituents are present in small quantities (F $2.1\,\%$, Na $1.0\,\%$, P $0.9\,\%$, S $0.6\,\%$, Mg $0.6\,\%$). Therefore, a substance typical for mineral component in bones, hydroxyapatite is likely present in the teeth among other substances.

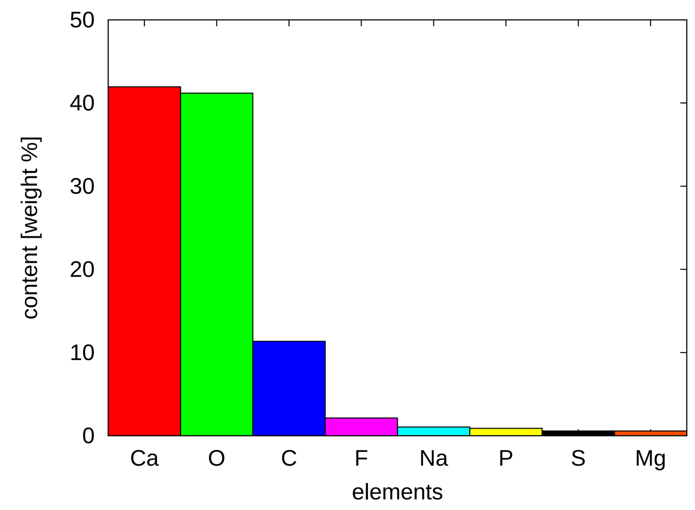

Figure: Elements concentrations obtained by EDS identify the presence of hydroxyapatite

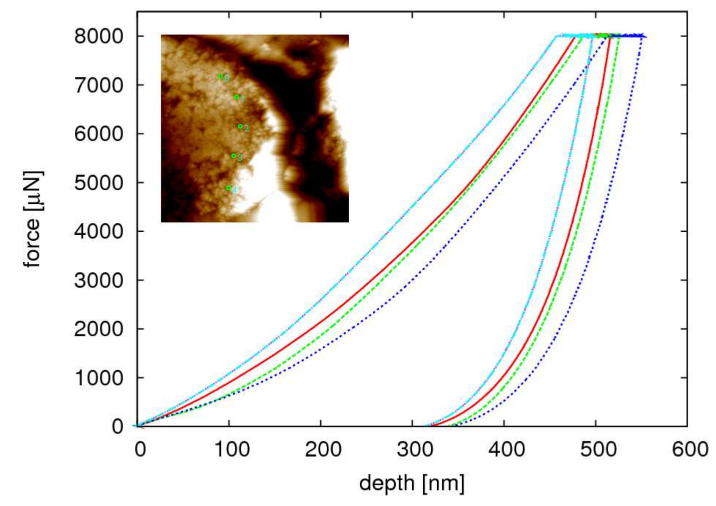

Figure: Five loading curves ($P_{max} = 8000\,\mu m$) on surface with roughness $R_a = 40\,nm$

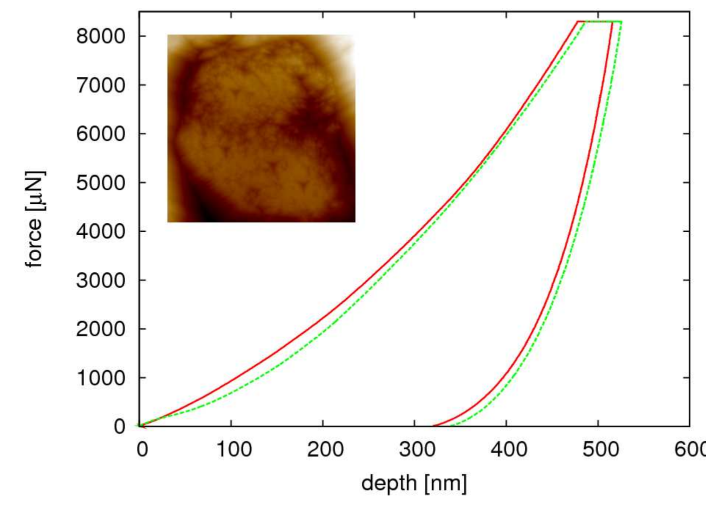

Figure: Two loading curves ($P_{max} = 8300\,\mu m$) on surface with roughness $R_a = 40\,nm$

The mechanical properties of two teeth leeches in cross-section were measured. Average values of reduced modules $E_r = 29.41 \pm 1.10\,GPa$, were obtained from 5 indents ($h_{max} \approx 150\,nm$, $R_a = 16\,nm$). In the next set of measurement values were $E_r = 27.02 \pm 4.03\,GPa$, where the 10 indent was performed ($h_{max} \approx 500\,nm$, $R_a \approx 40\,nm$). This values corresponds with the values of measurement on cortical bone and tooth dentin [6].

Conclusions

From the results of the nanoindentation it could be concluded that the mechanical properties of leech's tooth are independent on indentation depth. High-precision surface preparation allows to indent in small depths with a high accuracy. Another advantages can be seen in reduction of resin injection and also in the possibility to place more indents in the same area. Reduction of indents' size is very desirable because of the tiny cross section of the tooth surface, which is smaller than $1000\,\mu m^2$.

Mechanical properties and composition microanalysis of the teeth corresponding to biomaterials such as cortical bone or dentin [6] offers the assumption that it is possible that the tooth surface could be similar to enamel, as it is at higher animal species. Therefore, it would be beneficial to prepare samples for indentation in away that would enable the mechanical properties of the very surface of the tooth. This measurement would make possible to determine whether the outer layer is created by enamel layer or not and based on this information to create an accurate constitutive material model.

Acknowledgments

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References

[1] S.E. Blackshaw: *Comparative Biochemistry and Physiology (A), 105, 4*, pp:643-652, 1993

[2] E.J. Elliot, K.J. Muller: *Brain Research, 218, 1-2* pp:99-113, 1981

[3] Encyclopædia Britannica Online: *European medicinal leech*, http://www.britannica.com/EBchecked/topic/750132/leeching/, 2011

[4] M. Dudíková, D. Kytýř, T. Doktor, O. Jiroušek: *Chemické listy, 105*, pp:790-791, 2011

[5] W.C. Oliver, G.M. Pharr: *J. Mater. Res., 7(6)*, pp:1564-1583, 1992

[6] G. Lewis, J. S. Nyman: *J. of Biomedical Materials Research. Part B, Applied biomaterials, 87*, pp:288-303, 2008