NANOVEA®
A Better Measure.

Organic & Non Organic Shell Hardness Using Nanoindentation

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INTRO

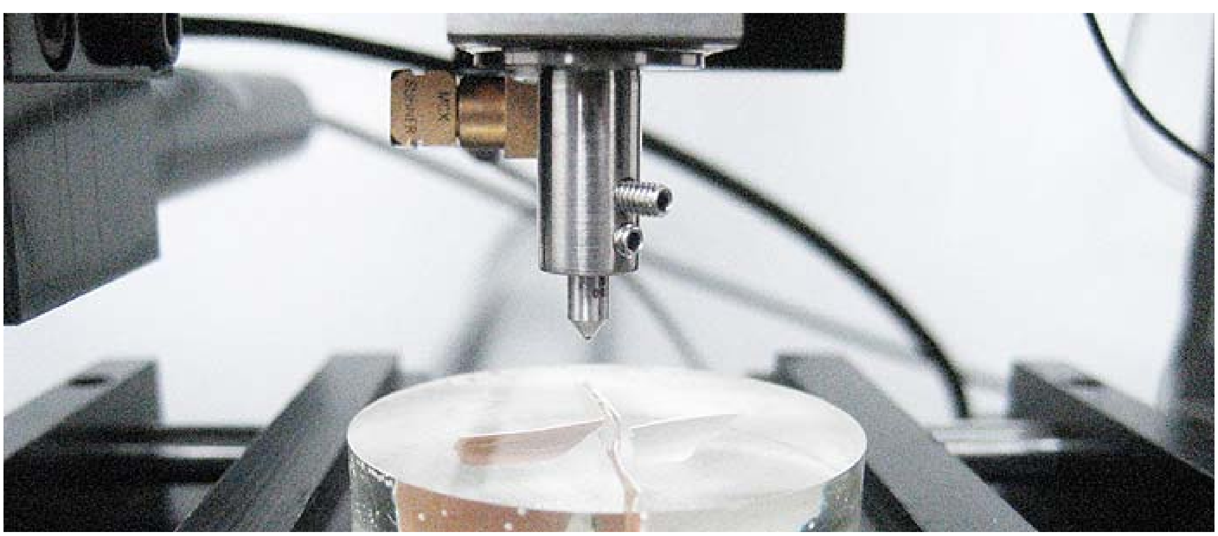
There have been many studies on egg shell hardness within food science research. But it is now the primary makeup of egg shells, Calcium Carbonate, that's of interest in the development of biomaterials. Synthetic biomaterials have emerged for bone regeneration, among many others, providing improved biocompatibility, biodegradability and mechanical properties. Calcium Carbonate has been widely studied as a composite with topics in Life Sciences, Material Sciences and Nanotechnology. Due to the wide range of applications with need for improved functional and structural properties it becomes critical that mechanical properties are known and controlled during the formulation of the composite makeup.

IMPORTANCE OF NANOINDENTATION FOR BIOMATERIALS

With many traditional mechanical tests (Hardness, Adhesion, Compression, Puncture, Yield Strength etc.), today's quality control environments with advanced sensitive materials, from gels to brittle materials, now require greater precision and reliability control. Traditional mechanical instrumentation fails to provide the sensitive load control and resolution required; designed to be used for bulk materials. As the size of material being tested became of greater interest, the development of Nanoindentation provided a reliable method to obtain essential mechanical information on smaller surfaces such as the research being done with biomaterials. The challenges specifically associated with biomaterials have required development of mechanical testing capable of accurate load control on extremely soft to brittle materials. In addition, multiple instruments are needed to perform various mechanical tests which can now be preformed on a single system. Nanoindentation provides a wide range of measurement with precise resolution at nano controlled loads for sensitive applications.

MEASUREMENT OBJECTIVE

In this application, the Nanovea Mechanical Tester, in Nanoindentation mode, is used to study the hardness and elastic modulus of an organic and a non-organic egg shell. The most critical aspect with Nanoidentation testing is securing the sample, here we took pieces of each sample and epoxy mounted leaving the edges exposed for testing.



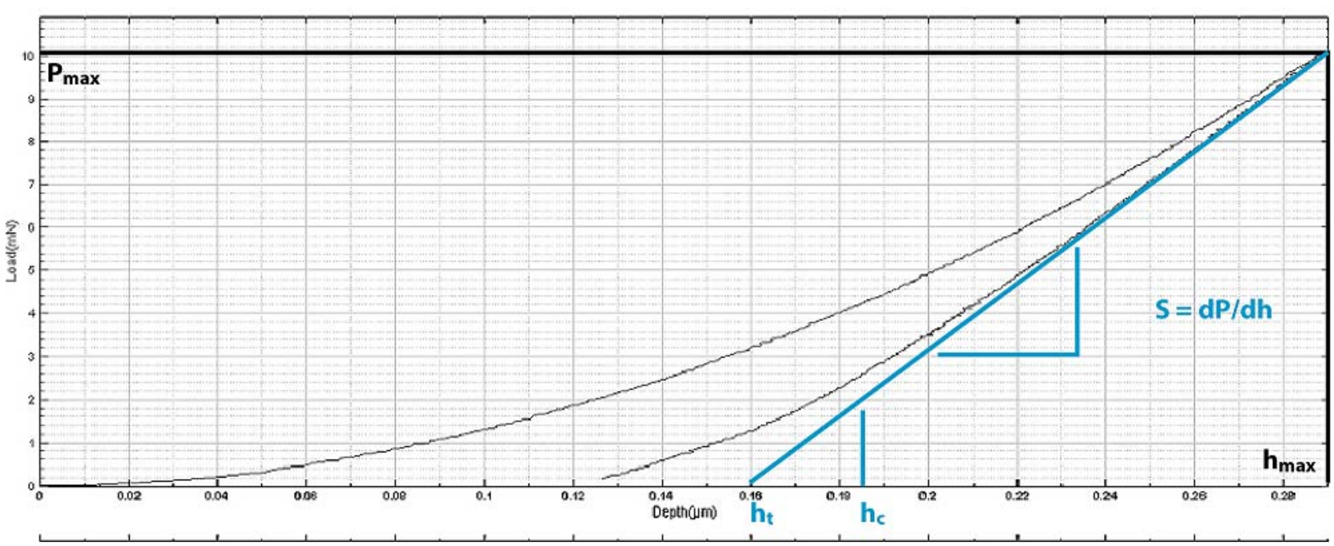
MEASUREMENT PRINCIPLE

Nanoindentation is based on the standards for instrumented indentation, ASTM E2546 and ISO 14577. It uses an already established method where an indenter tip with a known geometry is driven into a specific site of the material to be tested, by applying an increasing normal load. When reaching a pre-set maximum value, the normal load is reduced until complete relaxation occurs. The load is applied by a piezo actuator and the load is measured in a controlled loop with a high sensitivity load cell. During the experiment the position of the indenter relative to the sample surface is precisely monitored with high precision capacitive sensor. The resulting load/displacement curves provide data specific to the mechanical nature of the material under examination. Established models are used to calculate quantitative hardness and modulus values for such data. Nanoindentation is especially suited to load and penetration depth measurements at nanometer scales and has the following specifications:

Maximum displacement (Dual Range)	: 50 μm or 250μm
Depth Resolution (Theoretical)	: 0.003 nm
Depth Resolution (Noise Level)	: 0.05 nm
Maximum force	: 400 mN
Load Resolution (Theoretical)	: 0.03 μN
Load Resolution (Noise Floor)	: 1.5 μN

Analysis of Indentation Curve

Following the ASTM E2546 (ISO 14577), hardness and elastic modulus are determined through load/displacement curve as for the example below.



Hardness

The hardness is determined from the maximum load, P_{max}, divided by the projected contact area, A_c:

$$H = \frac{P_{\max}}{A_c}$$

Young's Modulus

The reduced modulus, E_r, is given by:

$$E_r = \frac{\sqrt{\pi}}{2}\frac{S}{\sqrt{A_c}}$$

Which can be calculated having derived S and A_C from the indentation curve using the area function, A_C being the projected contact area. The Young's modulus, E, can then be obtained from:

$$\frac{1}{E_r} = \frac{1-\nu^2}{E} + \frac{1-\nu_i^2}{E_i}$$

Where E_i and ν_i are the Young's modulus and Poisson coefficient of the indenter and ν the Poisson coefficient of the tested sample.

How are these calculated?

A power-law fit through the upper 1/3 to1/2 of the unloading data intersects the depth axis at h_t. The stiffness, S, is given by the slope of this line. The contact depth, h_c, is then calculated as:

$$h_c = h_{max} - \frac{3P_{max}}{4S}$$

The contact Area Ac is calculated by evaluating the indenter area function. This function will depend on the diamond geometry and at low loads by an area correction.

For a perfect Berkovich and Vickers indenters, the area function is $A_c=24.5h_c^2$ For Cube Corner indenter, the area function is $A_c=2.60h_c^2$ For Spherical indenter, the area function is $A_c=2\pi Rh_c$ where R is the radius of the indenter. The elastic components, as previously mentioned, can be modeled as springs of elastic constant E, given the formula: $\sigma = E\varepsilon$ where σ is the stress, E is the elastic modulus of the material, and ε is the strain that occurs under the given stress, similar to Hooke's Law. The viscous components can be modeled as dashpots such that the stress-strain rate relationship can be given as, $\sigma = \eta\frac{d\varepsilon}{dt}$
where σ is the stress, η is the viscosity of the material, and dε/dt is the time derivative of strain.

Since the analysis is very dependent on the model that is chosen. Nanovea provides the tool to gather the data of displacement versus depth during the creep time. The maximum creep displacement versus the maximum depth of indent and the average speed of creep in nm/s is given by the software. Creep may be best studied when loading is quicker. Spherical tip might be a better choice.

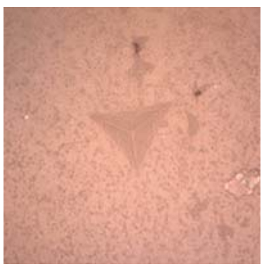
Other tests possible includes the following:

Puncture Resistance, Stress-Strain & Yield Stress, Fracture Toughness, Compression Strength, Fatigue testing and many others.

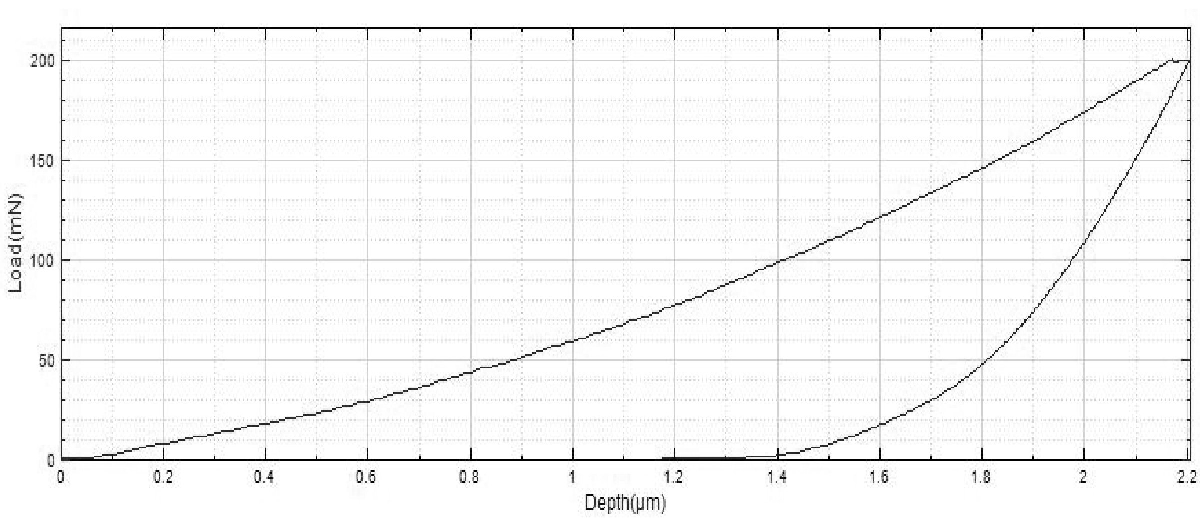
TEST CONDITIONS

Approach Speed	1µm/min
Contact Load	0.06 mN
Max Load	200mN
Unloading Rate	400mN
Creep (sec.)	20
Indenter Type	Berkovich

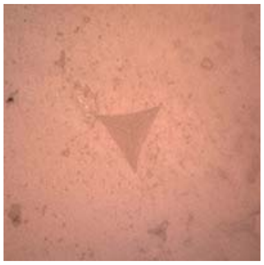
Non-Organic



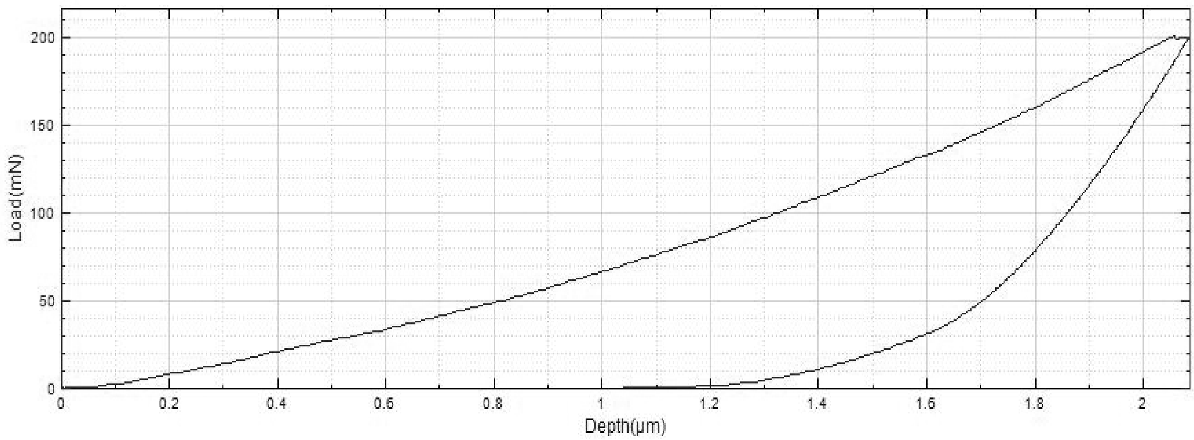
	H(v)	**H(gpa)**	**Modulus**	**Depth**
test 1	223.7	2.367	41.45	2166
test 2	213.6	2.261	41.10	2202
test 3	191.0	2.022	39.75	2308
average	**209.5**	**2.217**	**40.77**	**2225**
stdev	**16.71**	**0.177**	**0.894**	**73.46**



Organic



	H(v)	H(gpa)	Modulus	Depth
test 1	251.3	2.659	45.53	2052
test 2	247.7	2.621	51.20	2052
test 3	248.3	2.627	43.45	2074
average	**249.1**	**2.636**	**46.73**	**2059**
stdev	**1.929**	**0.020**	**4.010**	**12.67**



CONCLUSION

In conclusion, we have shown how the Nanovea Mechanical Tester, in Nanoindentation mode, provides precise measurement of the mechanical properties of egg shells. Organic fed chicken eggs were shown to be harder than the non organic fed. This demonstrates how the composition of biomaterials can affect its behavior for various applications.

Other tests possible with the same system could have included Fracture Toughness, Fatigue testing and many others. In addition, a puncture test with a specific geometry tip could be used to test the resistance of a full egg. In addition, Nanoindentation can be used to test fatigue and yield strength using multiple cycle loading. For soft applications such as soft gels, DMA "Dynamic Mechanical Analysis" can be used to measure viscoelastic properties including loss and storage moduli. The Nanovea nano module is ideal for this testing because it uses a unique feedback response to control precisely the load applied. Because the determination of contact point on soft materials is made through direct measurement of the load, the accuracy of the data is far superior to what is achievable with coil systems with no feedback loop. The large movement range of 50mm also allows a much larger range that is often necessary for bio materials such as brain samples. DMA is also measured very directly with no mathematical assumption because depth and load are directly measured at the point of contact in real time providing the highest accuracy and repeatability attainable. With the controlled XY stage, the same instrument can be used to measure scratch and marring properties or to measure coefficient of friction. Multi-pass wear is also a common test as it provides important information on the long term viability of some of the biomaterials. To learn more about Nanovea Nanoindentation.