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# Measurements of Elastic Properties of Biological Hydrogels using Atomic Force Microscopy

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**Abstract.** Biological hydrogels are widely used as extracellular environment for encapsulating and growing cells. Mechanical properties of hydrogels can influence cell function, mechanotransduction and cellular behavior such as growth, migration, adhesion, differentiation and morphology. Microenvironmental modulus of hydrogels dictates cell behavior and growth. The main purpose of the current study was to determine elastic modulus of two well-known hydrogels (Agarose and Gelatin Methacryloyl) using Atomic Force Microscope (AFM). The elastic modulus was calculated from force-deflection curve obtained by indenting an indenter in the direction normal to the plane of the gel surface. The elastic moduli of the prescribed gels were found to be strongly influenced by the level of concentration in the gel. Elastic modulus of 1% agarose and 2% agarose was found comparable with 10% GelMA and 20% GelMA, respectively. Results obtained from indentation experiments and those published in the literature revealed that AFM can be successfully and confidently used to determine elastic response of hydrogels in the solid state.

## 1. Introduction

Biological hydrogels closely mimic native extracellular matrices (ECMs) and possess physical properties closed to many soft biological tissues [1]. Water based hydrogels are unique biomaterials offering desirable characteristics such as biodegradability and porosity required for several applications in biomedical engineering [2]. Polymer based hydrogels are widely used in several biomedical devices, sensing technologies in medicine and are considered as safe and efficient drug carriers. Several applications of hydrogels in biomedical field were enabled by conducting extensive research on tuning their elastic properties from a few kPa to a few MPa. Tremendous research work has been carried out on developing hydrogels with control mechanical properties, chemical composition, and structure closed to native biological tissues [3]. One of the techniques for tuning the elastic properties of hydrogels is by varying the crosslinking density of the polymeric network. The elastic properties of hydrogels can be modified by varying the hydrogel concentration [4-7]. Mechanical and rheological properties of hydrogels reported in the published literature are elastic modulus, shear modulus, Poisson's ratio, viscosity, shear stress, loss modulus and storage modulus.



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Two well-known hydrogels used for electrophoresis or chromatography and variety of cell culture applications are Agarose and Gelatin Methacrylol (GelMA). Agarose based hydrogels are tested as a suitable biomaterial for its biocompatibility, viscoelastic properties mimicking tissue, and ease of casting into variety of complex shapes and sizes. GelMA hydrogels possesses essential properties of native extracellular matrix which encourage cells proliferation in GelMA-based scaffolds.

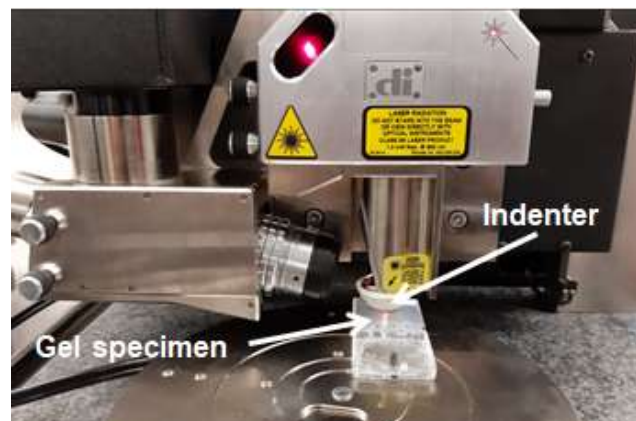
Determination of mechanical properties of hydrogels is importance for variety of applications in biomedical engineering. Several mechanical properties such as elastic modulus, strength, viscoelastic properties and rheological properties of hydrogels have been reported in the published literature [4, 8-11]. Stiffness of the hydrogel network significantly affect proliferation of cells and several other biological characteristics associated with gels [6,12]. The mentioned properties were measured using dynamic mechanical analyzer (DMA), nanoindentation, microtester, rheometer, and atomic force microscopy (AFM) indentation. Tension and compression tests were also used to measure the prescribed property of hydrogels in solid state [13,14]. One of the most commonly used techniques to study the mechanical behaviour of hydrogels is using an indenter pushed into the hydrogel. Detailed information on mechanical characterization of hydrogels is referred elsewhere [4].

AFM has successfully been applied to measure elastic properties of hydrogels [9,15], cells [15], cells scaffolds [16] and other soft elastic materials [17]. AFM provides advanced level of resolution maps for surface topography and can measure substrate stiffness, viscoelasticity and adhesion AFM with its continuous evolution has widely been used to assess mechanical properties of soft materials such as gel. Elastic properties of several hydrogels based soft materials measured using AFM are reported in a recent study [10]. Published literature on the mechanical properties presented inconsistent data on mechanical properties which make selection of a particular hydrogel for a specific application difficult. This study finds elastic modulus of agarose and GelMA hydrogels using AFM and compared the results.

## 2. Materials and Methods

To prepare agarose gels with 1% (w/v) and 2% (w/v) concentrations, agarose powder weight 1 gram and 2 grams were dissolved in 100 ml de-ionized water and boiled for approximately two minutes in the microwave. To form GelMA hydrogels, GelMA precursor solutions were prepared by dissolving freeze-dried GelMA in deionized water at 40°C. Aqueous solutions of 10% and 20% GelMA were prepared since this range of concentration has been found to have stiffness suitable for cell viability.

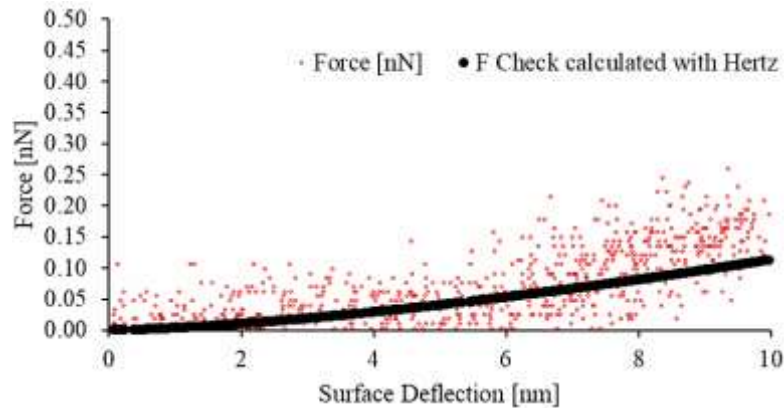
The Dimension 3100 AFM was used to find force-deflection. The equipment can measure the deflection of the sharp tip attached to a micron-sized cantilever. A DNP-10 tip B with a stiffness of 006 N/m and nominal tip radius of 20 nm was used to measure the elastic modulus of the surface Indentation experiment was repeated three times for each sample at different locations. All tests were carried out at room temperature (25°C). Experimental set up for measurement of elastic modulus is shown in figure 1.



**Figure 1.** Experimental set up for measurement of elastic modulus and surface topography.

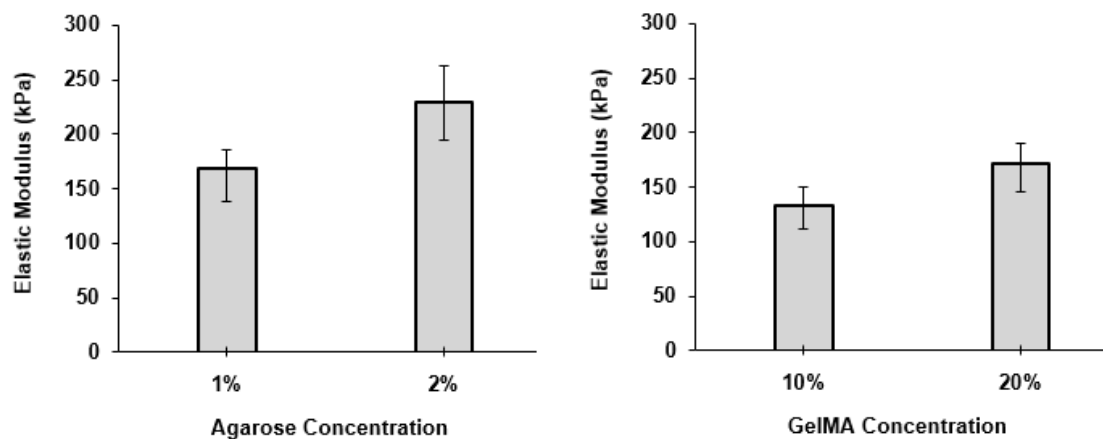
### 3. Results

The vertical displacement of the cantilever and its deflection (called force curve) were simultaneously recorded. The curve was obtained by moving the indenter in the direction normal to the plane surface of the sample. A typical force-deflection curve obtained from AFM measurements is shown in figure 2. The data points obtained were fit to a curve which was later used to determine elastic modulus of gel specimens.



**Figure 2.** Typical force-deflection graph obtained from AFM measurements.

Variation of elastic modulus for both types of gels and two different concentrations is shown in figure 3. The elastic modulus was significantly increased with increase in concentration. The elastic modulus was found to increase from an average value of 168 kPa to 230 kPa (35% increase) when concentration of agarose was increased from 1% to 2%. Similarly, the elastic modulus was noted to rise from an average value of 133 kPa to 171 kPa (38% increase) when concentration of GelMA was increased from 10% to 20%. The elastic moduli measured for 1% and 2% agarose was found comparable to 10% and 20% GelMA, respectively.



**Figure 3.** Variation of elastic modulus with gel concentration.

### 4. Conclusion

Atomic force microscopy (AFM) is used to determine elastic properties of agarose gel and Gelatin Methacryloyl of different concentrations. The elastic modulus increased with increase in level of concentrations in the gel. The study provided results on elastic modulus of two types of gels which are consistent with the published data AFM can be successfully used to determine elastic properties of hydrogels with porosity and water content. Further studies are suggested to determine elastic modulus of gels having concentrations more than studied in this paper

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