Application Note

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Mechanobiology of hydrogels

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Unraveling the mechanobiology of hydrogels with Pavone in tissue engineering, regenerative medicine, and drug delivery systems

by Optics11 Life

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The ability to design hydrogels with specific mechanical properties to direct cellular behavior and promote tissue regeneration represents a promising avenue for developing innovative therapies in bioengineering, regenerative medicine, and drug delivery systems. Here, we introduce the highthroughput mechanical screening platform Pavone for understanding the cell–hydrogel mechanics interactions and unlocking new therapeutic strategies.

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INTRODUCTION

In native tissues, cells interact with the surrounding extracellular matrix (ECM) through matrix-binding receptors forming a complex three-dimensional (3D) structure. They sense mechanical signals in their local environment by applying forces to their surrounding matrix, which induces biochemical signaling and leads to gene transcription, protein activation, and various cellular processes such as cell growth, migration, proliferation, and differentiation[1,2,3,4]. Understanding ECM composition and structure is critical for developing novel 3D cultures that predict biological mechanisms and therapeutic effects.

A versatile class of biomaterials known as hydrogels is central to tissue engineering, regenerative medicine, and drug delivery systems. Hydrogels are hydrophilic polymer networks that form a 3D structure[1,5]. They guide cell function and tissue development by mimicking the native ECM, regulating the delivery of biochemical compounds, and providing mechanical stimuli to the 3D cell construct. In addition, the ability of cells to interact mechanically with the surrounding matrix allows them to remodel hydrogels into tissue-like structures[5].

However, variations in the hydrogel's mechanical properties, such as strength, stiffness, density, composition, orientation, and viscoelastic, affect cellular activity[6]. Therefore, the main challenges in using hydrogels are controlling cell behavior, identifying mechanical triggers to initiate specific cell activities, and creating hydrogels with native tissue-like properties to promote tissue formation[7]. Besides, conventional methods for the mechanical characterization of hydrogels are complex. They require time-consuming sample preparations, which may lead to losing some of the mechanical properties native to the cells. To address the unmet needs in bioengineering, we introduce the Pavone Nanoindenter as a powerful method of mechanical characterization of cells, tissues, spheroids, organoids, hydrogels, scaffolds, and other biomaterials. In contrast to most classical methods, Pavone non-destructively characterizes a smaller sample volume and allows a more realistic profile of its nanoscale mechanical properties.

OPTICS11 LIFE NOVEL TECHNOLOGY

Pavone provides a mechanical screening platform for novel applications, such as disease modeling, drug screening and delivery, regenerative therapies, genetic medicine, and diagnosis of diseases. For instance, Pavone identifies the mechanical properties of complex and soft hydrogels, the mechanisms by which cells remodel hydrogels, the influence of hydrogel mechanical and structural properties on cell behavior, and the role of mechanical stimulation in cell-seeded hydrogels[8,9,10,11].

Pavone combines automated indentation mapping, imaging, fluorescence, and environment control. Compatible with up to 2 x 96-well plates, the instrument provides a screening-scale solution for large and multiday mechanical testing. Therefore, Pavone minimizes equipment requirements and allows for lower processing times and costs.

MECHANICAL CHARACTERISATION OF HYDROGELS

The Pavone performs static and dynamic indentation measurements. Static indentation measures the elasticity of elastic or elastic-plastic materials in terms of stiffness or Young's modulus E (Pa) and consists of loading, holding, and unloading indentation steps. Dynamic indentation characterizes viscoelastic material properties and consists of fast loading, holding, frequency sweep, and unloading steps. Dynamic mechanical analysis (DMA) applies an oscillatory force at a set frequency to the sample and reports changes in storage and loss moduli, E' and E'', and loss or damping factor $tan(\delta)$ (the ratio of $\frac{E''}{E'}$) as a function of frequency.

Here, we performed static and dynamic indentation measurements of the hydrogels with Pavone using a probe with a stiffness of 0.287 N/m and a tip radius of 47.0 μm. Each sample was immersed in dH20 before the nanoindentation experiments. The Young's modulus was measured as a function of three different hydrogel concentrations: 750, 1500, and 3000 µM. E', E'' and tan(δ) of the hydrogel (3000 μM) were measured as a function of frequency using DMA. Our results showed that the stiffness increased with the concentration of hydrogels (Figure 1). Besides, the E' of hydrogels decreased with frequency, and E'' and

tan(δ) are different from zero, confirming the viscoelastic properties of the material (Figure 2). This suggests that the viscoelastic characteristics of the hydrogel are very susceptible to the testing frequency, where a solid-to-liquid transition occurs around 12 Hz. It is relevant to highlight that the stiffness of hydrogels determine the cell fate and tissue development, while the dynamic changes in its viscoelastic behavior promote cell reorganization[12].

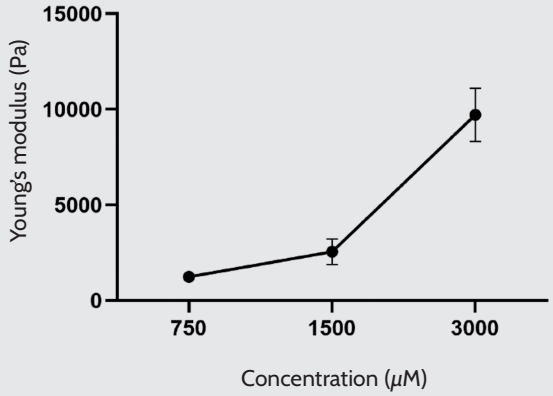

Figure 1

Young's Modulus of three different hydrogel concentrations: 750, 1500, and 3000 μM.

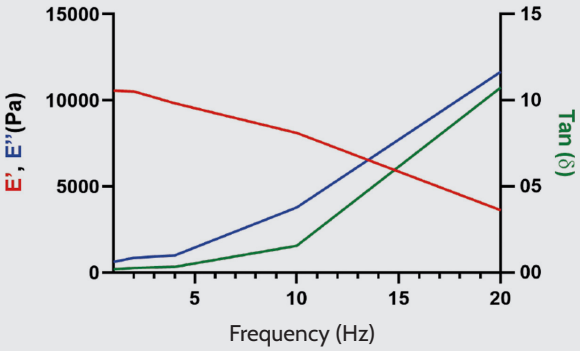

Figure 2

Dynamic mechanical analysis of hydrogel (3000 μM) as a function of frequency: storage modulus (E'), loss modulus (E''), and loss or damping factor $tan(\delta)$ ($\frac{E'}{E''}$).

4

CONCLUSION

Understanding the interaction between cells and their surrounding matrix in hydrogels is vital to developing bioengineering and regenerative medicine technologies. Furthermore, combining these next-generation hydrogels with Pavone will lead to a new mechanistic understanding of how mechanic forces impact normal physiology and disease. Therefore, translating the mechanics of hydrogels into clinical interventions will allow novel therapeutic approaches to several areas of medicine.

5

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