QUANTIFYING THE MECHANICAL STIFFNESS OF OVARIAN ECM HYDROGELS

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INTRODUCTION

Women who have cancer can often lose their reproductive abilities due to varied cancer treatments such as radiation or chemotherapies. For example, around 33% to 76% of the women with breast cancer experience symptoms of infertility following treatment [1]. The current solution to this clinical problem is cryopreservation, in which the eggs, also known as the oocytes, are removed from the woman prior to treatment and stored frozen until the treatment is done. The oocytes can then be thawed and returned back to the woman. However, this process often damages the oocytes in the freezing process and the thawed oocytes are not always viable [2].

An alternative approach is being proposed to restore fertility in women, which is to culture the oocytes on an extracellular matrix (ECM) hydrogel rather than freezing them. ECM is a network of protein fibers that exists on the outside of a cell and serves to support cell growth. A hydrogel is a three-dimensional gel made up of polymer fibers that can be used to culture cells. In this approach, immature oocytes are first isolated from the women prior to treatment. Then, they are cultured on a hydrogel made up of ECM extracted from ovarian tissues to obtain mature oocytes. The mature oocytes can subsequently be injected back into the women after their cancer treatments to restore fertility.

This process has several advantages. First, it bypasses the freezing process and therefore reduces the chance of damaging the oocytes. Second, the use of ovarian tissue ECM can better facilitate cell growth and development. Previous research has shown that the cells are the healthiest when grown in an environment similar to their native environment [3]. Since ECM is a naturally occurring fiber structure surrounding the cells, the use of ECM from ovarian tissues can best mimic the native tissue environment and better facilitate cell growth compared to non-ovarian tissue ECM fibers.

However, in order to successfully grow immature oocytes on the hydrogel, the mechanical stiffness of the hydrogel is extremely important. This is because different surrounding stiffness corresponds to different cell developmental stages. For example, immature oocytes usually reside on the outer cortex of the ovary, which is very stiff. As they mature, they will migrate towards the inner medulla of the ovary, which is soft [4]. Moreover, changing the amount of ECM in the hydrogel can alter the stiffness of the gel, which in turn can dramatically influence the development of the oocytes. Therefore, the stiffness of the hydrogel with different ECM concentrations must be quantified. This can be done by examining three variables: elasticity, viscosity, and strain. Strain corresponds to the deformation of a material, defined by Equation 1:

\[ \text{Strain} = \frac{\Delta L}{L} \]  

Where \( \Delta L \) is the change in length and \( L \) is the original length. When used in the context of measuring stiffness, strain corresponds to the amount of change in the length of a material required to break that material.

OBJECTIVE

The objective was to quantify the mechanical stiffness of the ovarian hydrogels with different ECM concentrations by examining the hydrogels’ elasticity, viscosity, and strain.

SUCCESS CRITERIA

Three success criteria must be met in order to deem the project as successful. First, there must be a significant difference in stiffness, as defined by the hydrogel’s viscosity and elasticity, between hydrogels with different ECM concentrations. Second, the hydrogel stiffness must not change over time, specifically over 40-minute duration. Third, the hydrogel must be able to withstand at least 5% deformation without significant changes in its stiffness. A significant change is defined as a greater than 5% change in either the viscosity or elasticity.

METHOD

Hydrogels with three different concentrations of ECM: 2, 5, and 10 mg/ml of ECM, were prepared. This was done by mixing the ECM extracted from porcine (pig) ovarian tissues with the appropriate volumes of phosphate buffer saline and sodium hydroxide, which served to dilute and neutralize the solution.

Rheology, which measures the flow of a material, was performed on the three ECM concentrations of hydrogels. First, 1.2 ml of the hydrogel solution prepared using the method described above was loaded onto the rheometer (AR1000) and the temperature was increased to 37 °C to induce gel formation. Then two rheological tests were run on the hydrogels: time and strain sweeps.

The time sweep measures the change in the hydrogel’s viscosity and elasticity overtime. A total of 6 samples for each ECM concentration of hydrogel was used. For each sample, the elasticity (storage modulus) and viscosity (loss modulus) data were collected at 15 equally spaced time points over a 40-minute duration. The hydrogels were tested under 5% strain and 1 rad/s frequency. An ANOVA was conducted for each time point to assess any significant difference in either the storage or loss moduli between the 2, 5, and 10 mg/ml ECM hydrogels. A p < 0.05 was deemed significant.

The strain sweep was used to identify the amount of deformation, or change in length, the hydrogel could withstand before breakage. A total of 5 samples for each ECM concentration of hydrogels was tested. Strains from 0.01 to 100% were tested under 1 rad/s frequency and the storage and loss
moduli were measured. The amount of strain required to cause a greater than 5% change in either the storage or loss moduli was noted.

RESULTS

All three ECM concentrations of hydrogels: 2, 5, and 10 mg/ml, showed a time-invariant stiffness after gelation. Gelation is when the hydrogel started to form from the original mixture solution, which can be identified by the initial increase in elasticity (storage modulus) during the first 10 minutes. After gelation, the elasticity of the hydrogel showed little to no change, indicating that the elasticity of the hydrogel is stable over time. In addition, there was a significant difference in elasticity between the three ECM concentrations of hydrogels, with p < 0.0001 for each time point (Fig. 1). The average elasticity for the 2, 5, and 10 mg/ml ECM hydrogels were 2.64 ± 0.23 Pa, 17.19 ± 0.98 Pa, and 46.58 ± 6.77 Pa respectively. This indicates that hydrogels with different ECM concentrations will have different stiffness.

The time sweep results for the viscosity (loss modulus) is not shown but shows the exact same trend. The average loss modulus for the 2, 5, and 10 mg/ml ECM hydrogels are 0.31 ± 0.03 Pa, 2.15 ± 0.28 Pa, and 7.63 ± 0.73 Pa respectively, with p < 0.0001 for each time point. The low loss modulus values mean that the hydrogels had low viscosity, which in turn is also an indication that gelation had successfully occurred and the hydrogels were stiff.

The strain sweep results showed that all three ECM concentrations of hydrogels experienced a less than 5% change in elasticity (storage modulus) for strain lower than 10% (Fig. 2).

The strain sweep results for viscosity is not shown but also shows a similar trend, with a less than 5% change in the loss modulus for strain less than 10%. Therefore, the strain sweep results indicate that there was little to no tearing or damage to the hydrogels for any strain or deformation less than 10%.

DISCUSSION

The three success criteria were met. First, a significant stiffness between hydrogels with different ECM concentrations was found, which shows that the amount of ECM in the hydrogel is an important determinant of the mechanical stiffness of the hydrogel. To facilitate the development of immature follicles to mature ones, a soft environment is preferred. Therefore, hydrogels with a lower ECM concentration is desirable. Second, all three ECM concentrations of hydrogels displayed a time-invariant stiffness after gelation. This proves that the hydrogels are stable and are capable of providing a consistent stiffness to the growing oocytes overtime. Lastly, the hydrogels were able to withstand strain greater than 5%. Deformation often occurs as a result of forces applied onto the hydrogels from the cells as they grow and migrate into the hydrogel. The average cell traction force is approximately 900 pN², which will produce a strain lower than 5% [5]. Therefore, being able to withstand a 10% strain demonstrates that the hydrogels have the sufficient stiffness to support cell growth without tearing or breakage.

One limitation of the experiment is the short time frame of the time sweep. The time sweep only tested the stability of the hydrogel over a 40-minute duration. However, oocyte cell culture usually requires weeks to months. Therefore, hydrogel stability after 40 minutes is not certain. Additional experiments, perhaps periodically checking the stability of the hydrogel during cell culture, are needed to determine the hydrogel’s long-term stability.

The next step is to culture the oocytes onto the hydrogels. Immature oocytes isolated from mouse will be cultured on either the 2 or the 5 mg/ml ECM hydrogels and the viability of the oocytes will be assessed overtime.

In conclusion, ovarian ECM hydrogel demonstrates sufficient mechanical stiffness to support oocyte growth. If oocyte culture is successful, the use of ovarian ECM hydrogels can be a promising solution to restore fertility in women.

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REFERENCES