

Determining Specific Heat Capacity of Physiologically Stiff Agarose Gel for Use in Chondrocyte Heat Generation Simulation

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DISCLOSURES: Dr. June owns stock in Beartooth Biotech and OpenBioWorks which were not involved in this study.

INTRODUCTION: Osteoarthritis is a widespread degenerative disease that affects millions of people in the United States alone.[1] The metabolism of chondrocytes affects cartilage health, and joint temperature increases during strenuous activity when the metabolic activity of chondrocytes may be altered. 4.5% agarose is used to simulate a physiologically stiff microenvironment for 3D culture mechanical stimulation of chondrocytes. In addition to agarose's physical similarity to cartilage stiffness, the thermal properties of agarose gel may affect the metabolism of chondrocytes during loading through viscoelastic heat generation. Understanding if chondrocytes can generate substantial heat during mechanical stimulation or other activity, and how this heat flows through agarose, is important for both basic chondrocyte biology and cartilage regeneration. Therefore, the **objective** of this study is to quantify the specific heat capacity of 4.5% low-temperature agarose gel using Differential Scanning Calorimetry (DSC).

METHODOLOGY: 4.5g of Type VIIA low-gelling temperature agarose powder (Sigma-Aldrich) was combined with 100mL of HPLC water and homogenized at 42°C to create 4.5% agarose gel. Liquified gel was pipetted into ten pre-manufactured aluminum DSC test pans (DSC Consumables) ~8mm in diameter. These filled test pans were then hermetically sealed using a combination of a crimped aluminum lid and cyanoacrylate and allowed to dry overnight. An additional five blank reference pans were also hermetically sealed with cyanoacrylate. The DSC operates by heating both a sample and reference pan at a constant temperature rate across a predefined range and comparing the difference in heat flow between the two. This difference is then used to calculate instantaneous specific heat capacity at any given temperature as follows:

$$C_p = \frac{E\Delta Q}{m}$$

where C_p is the specific heat capacity, ΔQ is the heat flow difference between the sample and reference pans, m is the mass of sample, and E is a calibration constant obtained by analyzing a material with known C_p . These experiments used sapphire powder (Al_2O_3) to calculate the calibration constant ($E = 0.02$). A constant heat ramp of 2°C/min from 10°C to 50°C was applied for each sample. After performing the sample heating regime using the DSC, heat flow plots were generated (Figure 1B). These plots were then used in tandem with the standard calibration value obtained from a sapphire calibration plot (Figure 1A), E , to calculate an average specific heat value of each sample across the entire temperature range (Figure 1C) ($C_{p,avg} = 2.85 \pm 0.04$ J/g°C).

RESULTS: The average agarose mass over ten test pans was 35.6 ± 1.5 mg, combined with an average of 7.8 ± 1.5 mg of cyanoacrylate to provide proper sealing. Empty control pans were sealed with an average of 8.7 ± 1.7 mg of cyanoacrylate, giving an average mass discrepancy of 0.87 ± 0.2 mg. Results of the DSC show a stable C_p over the applied temperature range, from ~ 2.78 J/g°C at 15°C to ~ 2.93 J/g°C at 50°C. Agarose heat capacity was ~ 2.87 J/g°C at 37°C, the nominal temperature in the human body. This value is significantly lower than water ($C_{p,water} \sim 4.18$ J/g°C) and could limit the conduction of heat through the cartilage.

DISCUSSION: From Figure 1D, while there is variance in pan (m1), agarose (m2), sealant mass (m3), and sealant mass difference (m4), only total pan mass (m1) had a correlation coefficient higher than 0.8, suggesting that the calculated specific heat was affected only by overall sample pan mass. Specific heat capacity dictates part of the thermal behavior of agarose during *in vitro* cyclical loading; knowing this will guide the development of heat transfer simulations of chondrocytes loaded within the agarose gel and their metabolic heat generation.

SIGNIFICANCE AND CLINICAL RELEVANCE: Understanding the thermal properties of *in vitro* cartilage models such as agarose gel is necessary to properly mimic *in vivo* cartilage for both heat transfer simulations as well as calculating heat generated from chondrocyte metabolic activity.

REFERENCES: [1] Hootman *et al*, Arthritis and Rheum 2006.

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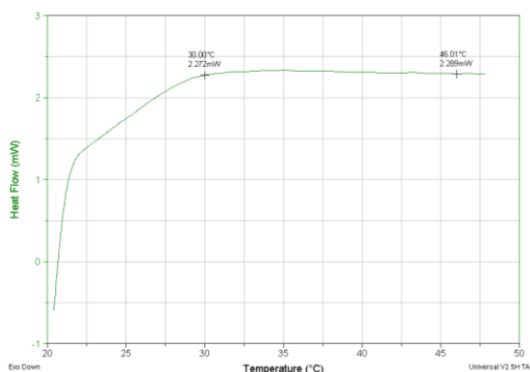


Figure 1A: Sapphire Calibration Curve

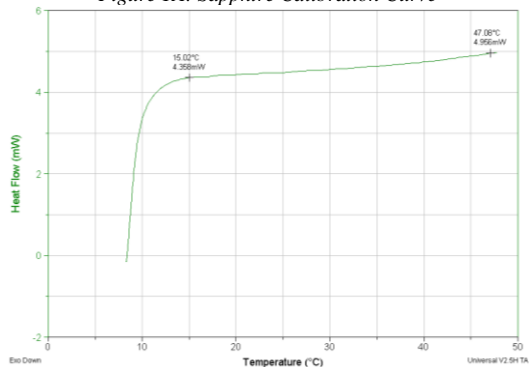


Figure 1B: Example Heat Flow Curve

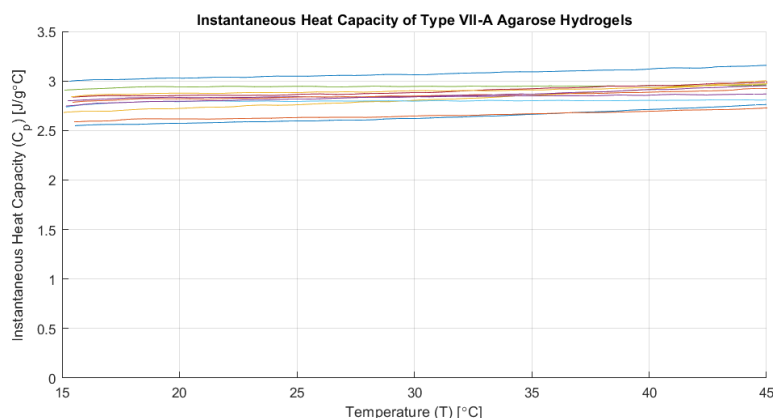


Figure 1C: Instantaneous Specific Heat Capacity of Type VII-A Agarose Hydrogels Shows Values Lower Than Water

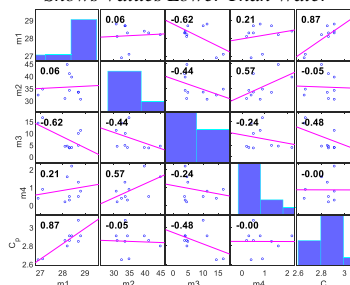


Figure 1D: Correlation Plot of Mass Data Shows Little Mass Dependence