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Avaliação da incerteza de medição da tensão superficial de esferoides celulares em testes compressivos usando a equação de Young-Laplace

Measurement uncertainty evaluation of cellular spheroids surface tension in compressing tests using Young-Laplace equation

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Resumo: A Medicina Regenerativa une a Biotecnologia, a Engenharia Tecidual e a Biometrologia na terapia com células tronco. Partindo de células tronco extraídas do paciente, implante autólogo, essas células são cultivadas e diferenciadas em outros tecidos, por exemplo, cartilagem articular. Essas células são reorganizadas em microesferas (esferoides celulares). Essas unidades teciduais são recombinadas em constructos de tecidos funcionais que podem ser implantados na região lesionada para sua regeneração. É necessária a caracterização biomecânica desses construídos para determinar se suas propriedades são similares às do tecido nativo. Nesse estudo foi realizada a modelagem do cálculo de incerteza da tensão superficial de esferoides celulares com o uso da equação de Young-Laplace. Obtivemos incertezas relativas da ordem de 10%.

Palavras-chave: Esferoides Celulares. Biometrologia. Engenharia Tecidual. Tensão Superficial.

Abstract: Regenerative Medicine comprises the Biotechnology, Tissue Engineering and Biometrology for stem cell therapy. Starting from stem cells extracted from the patient, autologous implant, these cells are cultured and differentiated into other tissues, for example, articular cartilage. These cells are reorganized into microspheres (cell spheroids). Such tissue units are recombined into functional tissues constructs that can be implanted in the injured region for regeneration. It is necessary the biomechanical characterization of these constructed to determine if their properties are similar to native tissue. In this study was carried out the modeling of the calculation of uncertainty of the surface tension of cellular spheroids with the use of the Young-Laplace equation. We obtained relative uncertainties around 10%.

Keywords: Cellular Spheroids. Biometrology. Tissue Engineering. Surface Tension.

1. INTRODUCTION

Regenerative therapies seek to improve the condition of patients affected by injuries that limit or prevent the normal activities of a healthy individual. One of the most promising regenerative therapies is the cartilage implant in injured joints. For these implants to result in a permanent cure, it is necessary to determine procedures and techniques to provide these implanted tissues similar characteristics to or better than the native tissue, for instance its resistance to compressive forces. Thus, the determination of the biomechanical properties of these constructs is fundamental for the success of these therapies. Metrological traceability can provide a more precise characterization of the cellular spheroids properties. Comparative research guarantees only qualitative results, but for comparisons with international results of researches we need a quantitative characterization of biomechanical properties of these constructs, it is mandatory a quality control in these results [1, 2]. The determination of the uncertainties associated with the measurements will lead to quantitative results of the biophysical properties analyzed. Therefore, it will be possible to attest that the conditions exhibited by these new 3D constructs are compatible with native tissues and that the techniques of cellular spheroid biofabrication and their subsequent bioprinting [3, 4, 5], this will contribute decisively to provide precise parameters to compare the different biofabrication techniques. In this study, we present a measurement uncertainty evaluation model for the measurement results to cellular spheroids surface tension in microcompression tests. Compressive tests were performed on a parallel plates micro tester where the spheroid is placed and a micronewton scale compression is exerted. The resulting strain is analyzed by the Young-Laplace equation [6, 7, 8] which gives the surface tension resulting from the applied pressure. The uncertainties involved were obtained by the classical model and validated by the Kragten method. The final values of the relative uncertainties were less than 10%, which means an excellent result in biometrology.

2. COMPRESSION TECHNIQUE

A cellular spheroid is placed between the parallel plates device and a compression force is exerted on it by a tungsten (W) microbeam (figure 1 and 2). The force measurement was performed with an analytical balance.

Figure 1. Cellular spheroid in the mechanical tester.

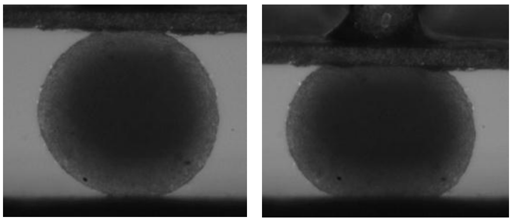
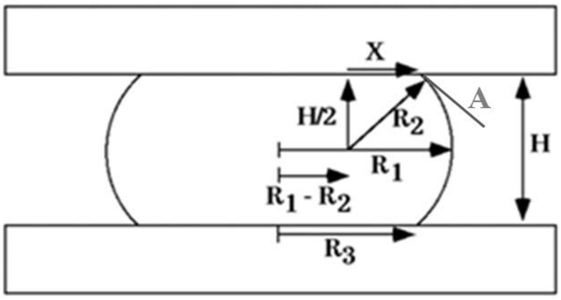


Figure 2. Cellular spheroid compressed between two parallel plates scheme (adapted from [9]).



The surface tension, under these conditions, can be obtained directly from the Young-Laplace equation (1).

$$T = \frac{F}{\pi R_3^2\left(\frac{1}{R_1} + \frac{1}{R_2}\right) - 2\pi R_3 sinA} \quad (1)$$

Where,

T → cellular spheroid surface tension.

F → applied force.

R_1 → equatorial curvature radius.

R_2 → border curvature radius.

R_3 → circular contact area radius.

A → angle (rad).

The A angle is the angle between the up spheroid surface and the parallel plate (figure 2). This angle can be rewritten in principal radii terms according to equation (2).

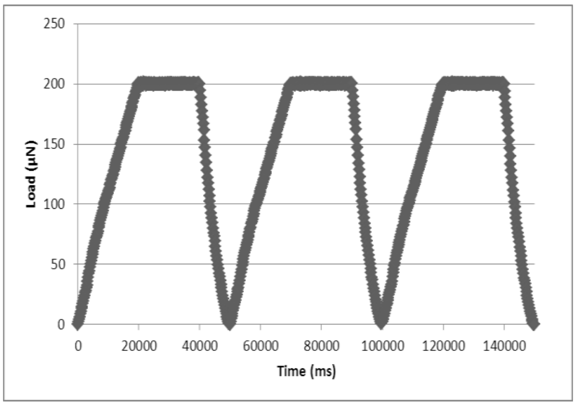
$$sin(A) = \frac{(R_3+R_2-R_1)}{R_2} \quad (2)$$

Substituting (2) into (1) we have,

$$T = \frac{F}{\pi R_3^2(\frac{1}{R_1}+\frac{1}{R_2}) - \frac{2\pi R_3}{R_2}(R_3+R_2-R_1)} \quad (3)$$

Thus (3) not depends explicitly of A angle. The compressing test is performed by setting the equipment with the chosen parameters and applying a 25% maximum displacement of the initial non-loaded spheroid diameter, recording the maximum force values in 3 up to 5 repetitions per cycle, figure 3.

Figure 3. A typical load test in an elastic (non-biological) sample.



3. UNCERTAINTY MODELLING

The identified uncertainty contributions u_y are shown in the Ishikawa diagram, figure 4. The combined uncertainties [10] are obtained from the input relative uncertainties by the general equation (4),

$$\frac{u_y}{y} = \sqrt{\sum_i \left(\frac{u_i}{xi}\right)^2} \quad (4)$$

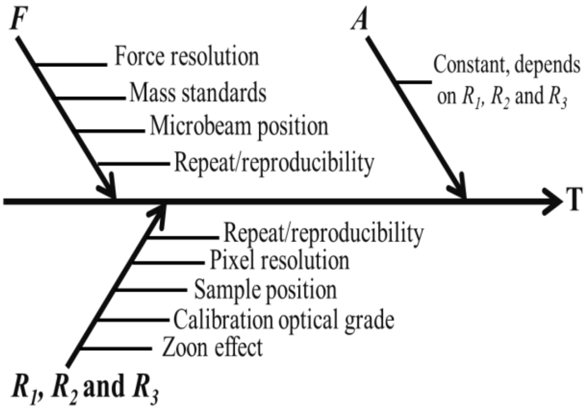
Where,

$u_y \rightarrow$ uncertainties ($y = F, R_1, R_2$ or R_3).

$u_i \rightarrow$ input uncertainties from Ishikawa diagram.

$x_i \rightarrow x_i$ modulus related to u_i.

Figure 4. Ishikawa diagram with the entrance uncertainty quantities.



Each of the uncertainty components is obtained from the product of the sensitivity coefficient c_y by the value of the uncertainty u_y of the input magnitude and the combined uncertainty to the surface tension $u_c(T)$ is given by the sum of modulus of uncertainty components (5)

$$u_c(T) = \sqrt{\sum c_y{}^2 u_y^2} \quad (5)$$

with $y = F, R_1, R_2$ or R_3

The coefficients of sensitivity are given by,

$$c_y = \frac{\partial T}{\partial y} \quad (6)$$

Here we will show the equation for the simple form (1), without the $sinA$ explicit equation (2), thus,

$$c_F = \frac{1}{\pi R_3^2(\frac{1}{R_1}+\frac{1}{R_2}) - 2\pi R_3 sinA} \quad (7)$$

$$c_{R_1} = \frac{\pi F\frac{R_3^2}{R_1^2}}{\left[\pi R_3^2(\frac{1}{R_1}+\frac{1}{R_2}) - 2\pi R_3 sinA\right]^2} \quad (8)$$

$$c_{R_2} = \frac{\pi F\frac{R_3^2}{R_2^2}}{\left[\pi R_3^2(\frac{1}{R_1}+\frac{1}{R_2}) - 2\pi R_3 sinA\right]^2} \quad (9)$$

$$c_{R_3} = \frac{-2\pi F\left(R_3(\frac{1}{R_1}+\frac{1}{R_2}) - sinA\right)}{\left[\pi R_3^2(\frac{1}{R_1}+\frac{1}{R_2}) - 2\pi R_3 sinA\right]^2} \quad (10)$$

$$c_A = \frac{2\pi R_3 F}{\pi R_3^2(\frac{1}{R_1} + \frac{1}{R_2}) - 2\pi R_3 sinA} \quad (11)$$

4. RESULTS

Using the following measurements results (obtained from a cartilage spheroid sample),

$A =$ 35.5° or 0.6196 rad

$T =$ 0.621 N/m

$R_1 =$ 245.9 µm

$R_2 =$ 175.8 µm

$R_3 =$ 172.1 µm

$F =$ 174.1 µN

with the uncertainties (figure 4) and traceability to the kilogram [11, 12] and meter [13] we have the force entrances uncertainties values (table 1).

Table 1. Force uncertainties values.

Entrance uncertainty	Uncertainty value
Force resolution	0.577 µN
Mass standards [11]	8 µg
Microbeam position	2.009 µN
Repeatability	0.387 µN
Reproducibility	0.516 µN

and applying in the equation (4) to the force uncertainty case we have,

$$u(F) = 2.203 \ \mu\text{N} \quad (12)$$

Performing the same procedure to the radii R_1, R_2 and R_3 we have (table 2),

Table 2. Radii uncertainties values.

Entrance uncertainty	Uncertainty value
Zoon effect	0.189 µm
Sample position	0.051 µm
Optical grade calibration [13]	0.060 µm
Pixel resolution	0.866 µm
Repeatability	0.387 µm
Reproducibility	0.516 µm

In the same way applying in (4) we have,

$$u(R_1) = 1.081 \ \mu\text{m} \quad (13)$$

$$u(R_2) = 0.774 \ \mu\text{m} \quad (14)$$

$$u(R_3) = 0.758 \ \mu\text{m} \quad (15)$$

$u(A)$ is not shown here because the uncertainties calculations was performed with the explicit equation for the A angle (2).

To obtain the surface tension expanded uncertainty we need to determine the k coverage factor which depends on effective degrees of freedom. In this case effective calculated degrees of freedom is,

$$\nu_{eff} = 9.444 \quad (16)$$

Considering a Gaussian distribution this value correspond to a coverage factor,

$$k = 2.262 \quad (p = 95\%) \quad (17)$$

Putting the results (12) (13) (14) and (15) in (5) we obtain the spheroid surface tension combined uncertainty,

$$u_c(T) = 0.0166 \ \text{N/m} \quad (18)$$

The expanded uncertainty is

$$U(T) = k\, u_c(T) \quad (19)$$

Putting (17) and (18) in (19) we have,

$$U(T) = 0.0377 \cong 0.038 \ \text{N/m} \quad (20)$$

The result to the spheroid surface tension in this approach is,

$$T = 0.621 \ \text{N/m} \pm 0.038 \ \text{N/m} \quad (21)$$

or

$$T = (0.621 \pm 0.038) \ \text{N/m} \quad (22)$$

Corresponding to 6.1 % in the relative expanded uncertainty. We verify the modelling and calculations using the Kragten method to uncertainty and it was obtained the same results,

$$u_c(T)_{Kragten} = 0.0375 \ \text{N/m} \quad (23)$$

When we compare the finals uncertainties without approximations to both approaches we have a relative difference of -5×10^{-5}, showing the agreement in our results.

5. CONCLUSIONS

We conclude that by considering all input identifying uncertainties and applying the Kragten approach or the classical model, we achieve a relative expanded uncertainty less them 10% in the presented case. These values are smaller than the variability of cellular spheroid properties. Therefore, this uncertainty calculation model can be used to determine the biomechanical properties of cellular spheroids. This modeling was performed for cellular spheroids with less than 2% sphericity. The next steps are to evaluate the sphericity effect on measurement uncertainty because are common samples with sphericity greater than 2% and to apply the same approach for the Young's modulus uncertainties calculation.

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