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

Anderson Beatrici¹,², Leandra Santos Baptista²,³ and José Mauro Granjeiro²

¹ National Institute of Metrology, Quality and Technology (Inmetro) - Scientific and Technological Metrology Division (Dimci), RJ, Brazil.
² National Institute of Metrology, Quality and Technology (Inmetro) - Division of Metrology Applied to Life Sciences (Dimav), RJ, Brazil.
³ Multidisciplinary research center in Biology - Numpex-Bio, Federal University of Rio de Janeiro, Campus of Duque de Caxias; Graduate Program in Biotechnology, Brazil.

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 about 10%.

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 micro compression tests. Compressive tests were performed on a parallel plates micro tester where the spheroid is placed and a micro newton 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, uncompressed (a), compressed (b).](image)

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

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

\[
T = \frac{F}{\pi R_3^2 \left( \frac{1}{R_1} + \frac{1}{R_2} \right) - 2 \pi R_3 \sin A}
\]

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).

\[
\sin(A) = \frac{(R_2 + R_2 - R_1)}{R_2}
\]
Substituting (2) into (1) we have,

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

(3)

Thus the equation (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. This maximum displacement was chosen because in this limit the spheroid compression do not able to deform permanently the biological samples.

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

### 3. Uncertainty modeling

The identified uncertainty contributions are shown in the Ishikawa diagram, figure 4. The \( A \) angle uncertainty contributions was taking account in terms of the three main curvature radius as shown in equation (2). The curvature radius and force uncertainty contributions were obtained directly by the calibrations certificates and measurement results analyses, as well as by mathematical simulations.

![Figure 4. Ishikawa diagram with the entrance uncertainty quantities.](image)

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 \( u_s(T) \) is given by the sum of modulus of uncertainty components (4)
\[ u_c(T) = \sqrt{\sum c_y^2 u_y^2} \]  
(4)

with \( y = F, R_1, R_2 \) or \( R_3 \).

The coefficients of sensitivity are given by,
\[ c_y = \frac{\partial T}{\partial y} \]  
(5)

Here we will show the equation for the simple form (1), without the \( \sin A \) explicit equation (2), thus,

\[ C_F = \frac{1}{\pi R_3^2 \left( \frac{1}{R_1} + \frac{1}{R_2} \right) - 2\pi R_3 \sin A} \]  
(6)

\[ C_{R_1} = \frac{\pi F R_3^2}{\left[ \pi R_3^2 \left( \frac{1}{R_1} + \frac{1}{R_2} \right) - 2\pi R_3 \sin A \right]^2} \]  
(7)

\[ C_{R_2} = \frac{\pi F R_3^2}{\left[ \pi R_3^2 \left( \frac{1}{R_1} + \frac{1}{R_2} \right) - 2\pi R_3 \sin A \right]^2} \]  
(8)

\[ C_{R_3} = \frac{-2\pi F \left( R_3 \left( \frac{1}{R_1} + \frac{1}{R_2} \right) - \sin A \right)}{\left[ \pi R_3^2 \left( \frac{1}{R_1} + \frac{1}{R_2} \right) - 2\pi R_3 \sin A \right]^2} \]  
(9)

\[ C_A = \frac{2\pi R_3 F \cos A}{\pi R_3^2 \left( \frac{1}{R_1} + \frac{1}{R_2} \right) - 2\pi R_3 \sin A} \]  
(10)

4. Results

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

\begin{align*}
A &= 35.5^\circ \text{ or } 0.6196 \text{ rad} \\
T &= 0.621 \text{ N/m} \\
R_1 &= 245.9 \text{ \( \mu \)m} \\
R_2 &= 175.8 \text{ \( \mu \)m} \\
R_3 &= 172.1 \text{ \( \mu \)m} \\
F &= 174.1 \text{ \( \mu \)N}
\end{align*}

with the uncertainties (figure 4) and traceability to the kilogram [11, 12] and meter [13, 14] we have the force entrance uncertainties values (table 1).

| Entrance uncertainty | Uncertainty value |
|-----------------------|-------------------|
| Force resolution [12] | 0.565 \( \mu \)N  |
| Mass standards [11]   | 0.048 \( \mu \)N  |
| Microbeam position [14]| 1.256 \( \mu \)N  |
| Repeatability         | 0.626 \( \mu \)N  |
| Reproducibility       | 0.447 \( \mu \)N  |
and applying in the equation (4) and (5) to the force uncertainty case we have,

$$u(F) = 1.578 \, \mu N$$

(11)

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

**Table 2. Radii uncertainties values to $R_i$ case.**

| Entrance uncertainty       | Uncertainty value |
|----------------------------|-------------------|
| Zoon effect                | 0.223 $\mu$m      |
| Sample position [14]       | 0.089 $\mu$m      |
| Optical grade calibration [13] | 0.060 $\mu$m |
| Pixel resolution           | 0.577 $\mu$m      |
| Repeatability              | 0.670 $\mu$m      |
| Reproducibility            | 0.894 $\mu$m      |

In the same way applying in (4) and (5) we have,

$$u(R_1) = 1.282 \, \mu m$$

(12)

$$u(R_2) = 1.138 \, \mu m$$

(13)

$$u(R_3) = 1.136 \, \mu m$$

(14)

$u(A)$ 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,

$$\nu_{eff} = 34.04$$

(15)

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

$$k = 2.032 \quad (p = 95\%)$$

(16)

Putting the results (11) (12) (13) and (14) in (4) we obtain the spheroid surface tension combined uncertainty,

$$u_c(T) = 0.02014 \, N/m$$

(17)

The expanded uncertainty is

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

(18)

Putting (16) and (17) in (18) we have,

$$U(T) = 0.040937 \approx 0.041 \, N/m$$

(19)

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

$$T = 0.621 \, N/m \pm 0.041 \, N/m$$

(20)

or

$$T = (0.621 \pm 0.041) \, N/m$$

(21)

Corresponding to 5.59 % in the relative expanded uncertainty. We verify the modeling and calculations using the Kragten method to uncertainty and it was obtained the same results,

$$U(T)_{Kragten} = 0.040941 \, N/m$$

(22)

When we compare the finals uncertainties without approximations to both approaches we have a difference of $-2.6 \times 10^{-6} \, N/m$, 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 than 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 there 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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