

Quantitative MRI Relaxation Parameters for Hypoxia Assessment in 3D Cancer Cell Spheroids



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Introduction

- Tumor hypoxia occurs when parts of a tumor have low oxygen levels. It is associated with treatment resistance and poorer clinical outcomes but is difficult to measure non-invasively.
- Quantitative MRI relaxation parameters may reflect hypoxia-related microenvironmental changes, but they are not specific to oxygen level alone.
- 3D cancer spheroids grown in microfluidic chips provide a controlled model where hypoxia develops naturally as spheroid size increases.

Can quantitative MRI relaxation parameters identify biologically validated hypoxia in 3D tumor spheroids?

Methods

- Microfluidic chip model:** HCT116 cells were seeded at four concentrations to generate spheroids of 400–1050 μm . Included 57 spheroid-containing chips:

18 chips at 400 μm ,
18 chips at 650 μm ,
10 chips at 800 μm ,
and 11 chips at 1050 μm .
- MRI acquisition:** Multi-parametric MRI was performed on a clinical 3T Siemens scanner using sequences for T_1 , T_2 , and T_2^* mapping. T_2' was derived from T_2 and T_2^* values.
- Image analysis:** Quantitative MRI maps were generated, and relaxation values were extracted from chip-level regions of interest.
- Molecular validation:** Western blotting was used to measure CA-IX protein expression, normalized to β -actin, as a biological marker of hypoxia.
- Statistical analysis:** Group differences were assessed using Kruskal–Wallis tests with post-hoc Dunn's comparisons, and monotonic size-dependent trends were evaluated using Kendall tau correlation.

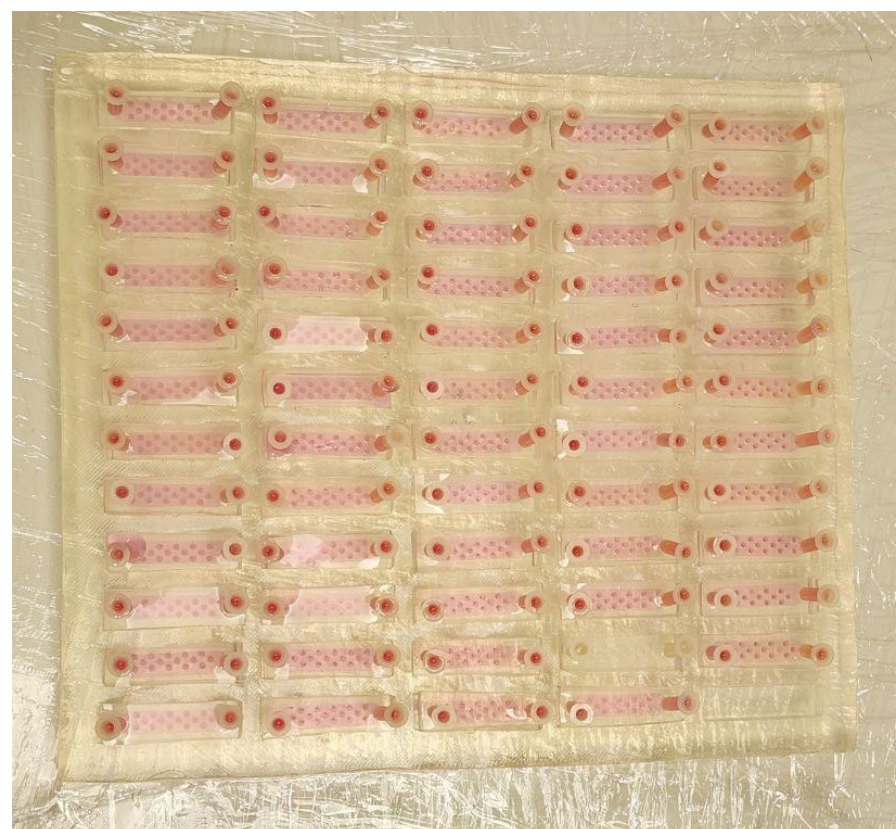


Figure 1. Microfluidic chip used for 3D spheroid culture.

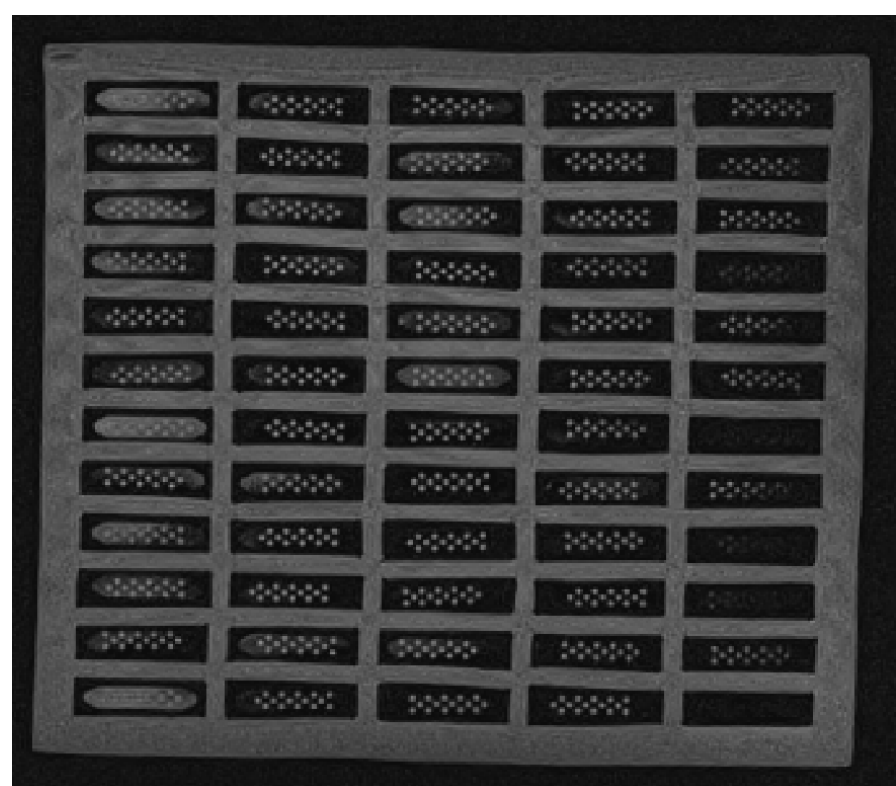


Figure 2. Representative T_2 -weighted MRI slice of the microfluidic chip array.

Results

- CA-IX/ β -actin expression increased significantly with spheroid diameter, confirming greater hypoxia in larger spheroids (Kruskal–Wallis, $H = 8.19$, $p = 0.042$; Kendall $\tau = 0.75$, $p = 0.002$).
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- Figure 3. (a) Representative spheroid images showing increasing spheroid diameter from 400 to 1050 μm . (b) Western blot analysis of pre-MRI samples showing increased CA-IX expression with spheroid diameter. β -actin was used as the loading control.
- T_2 relaxation time decreased from 291.0 ± 36.7 ms in 400 μm spheroids to 235.5 ± 31.6 ms in 1050 μm spheroids, showing the strongest MRI association with increasing hypoxia.

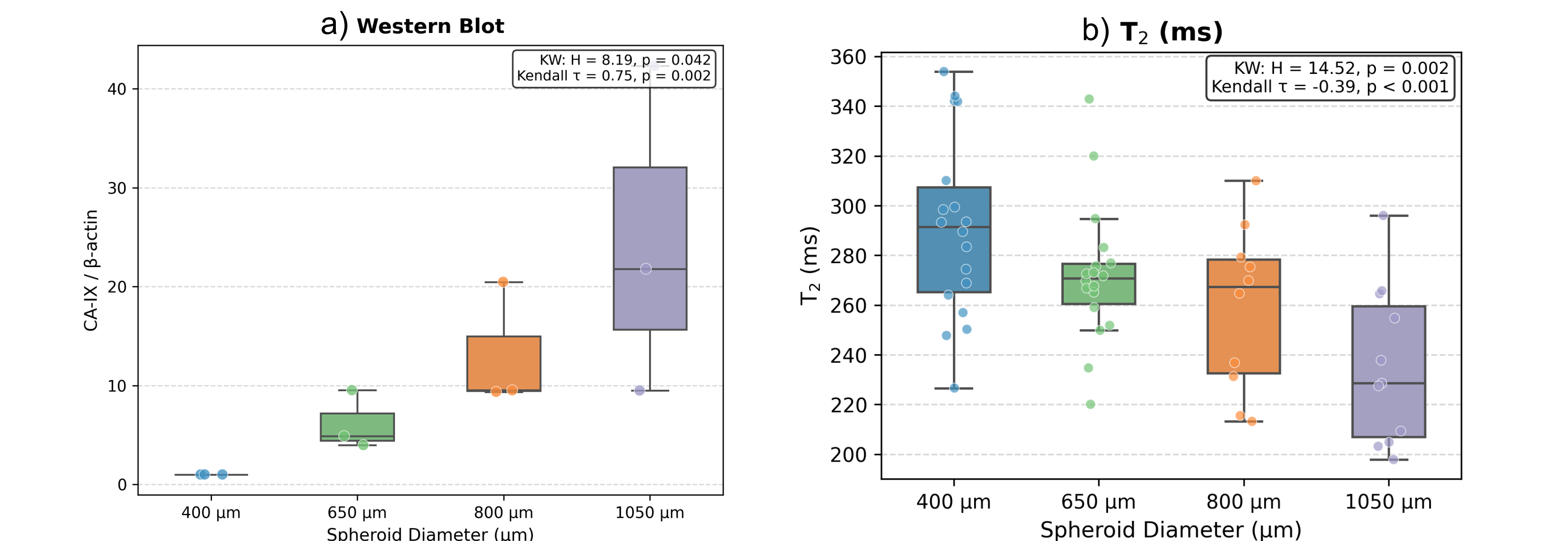


Figure 4. (a) Western blot quantification showed a significant increase in CA-IX/ β -actin with spheroid diameter. (b) T_2 mapping showed a significant decrease in T_2 relaxation time with increasing spheroid diameter.

- The opposite trends of CA-IX and T_2 support an inverse relationship between biologically validated hypoxia and T_2 relaxation in this spheroid model.

MRI parameter	Kruskal–Wallis test	Kendall τ trend test	Interpretation
T_2	$H = 14.52$, $p = 0.002$	$\tau = -0.39$, $p < 0.001$	Significant decrease
T_1	$H = 7.56$, $p = 0.056$	$\tau = -0.17$, $p = 0.099$	Trend only
T_2^*	$H = 1.30$, $p = 0.730$	$\tau = -0.07$, $p = 0.482$	No
T_2'	$H = 0.91$, $p = 0.822$	$\tau = -0.04$, $p = 0.677$	No

Table 1. Statistical summary of MRI relaxation parameters across spheroid-size groups. Only T_2 showed both a significant group difference and a significant monotonic decrease with spheroid diameter.

Discussion

- The inverse relationship between CA-IX and T_2 suggests that T_2 is sensitive to hypoxia-related changes in the spheroid microenvironment.
- T_2 may reflect combined effects of increasing cellular density, reduced free water mobility, and structural changes that occur as spheroids become larger and more hypoxic.
- The absence of significant T_2^* and T_2' changes may be related to the in-vitro model, which lacks blood flow and deoxyhemoglobin-based susceptibility effects seen in vivo.
- This controlled spheroid model reduces biological complexity and allows MRI parameters to be tested directly against molecular hypoxia validation.
- These findings provide a foundation for future studies using multiparametric MRI and machine learning to develop non-invasive hypoxia maps.

Conclusion

- Microfluidic 3D spheroids provided a controlled in-vitro model for studying diffusion-limited tumor hypoxia.
- Western blot validation confirmed that CA-IX expression increased with spheroid size, supporting the presence of greater hypoxia in larger spheroids.
- Among the MRI relaxation parameters evaluated, T_2 showed the strongest and most significant association with biologically validated hypoxia.
- T_1 showed only a weak trend, while T_2^* and T_2' did not demonstrate significant sensitivity in this model.
- These findings support T_2 relaxation mapping as a promising MRI-based approach for future non-invasive hypoxia assessment.

This study identifies T_2 relaxation as the most promising MRI parameter for detecting biologically validated hypoxia in 3D tumor spheroids.

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More Info
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References

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