



HAL
open science

3D MRI characterization of 3D printed tumor tissue models using a plastronic MR-Bioreactor: Preliminary results.

Jean-Lynce Gnanago, Tony Gerges, Laura Chastagnier, Emma Petiot, Vincent Semet, Philippe Lombard, Marquette Christophe, Michel Cabrera, Simon A. Lambert

► To cite this version:

Jean-Lynce Gnanago, Tony Gerges, Laura Chastagnier, Emma Petiot, Vincent Semet, et al.. 3D MRI characterization of 3D printed tumor tissue models using a plastronic MR-Bioreactor: Preliminary results.. Joint Annual Meeting ISMRM-ESMRMB & ISMRT 31st Annual Meeting, International Society for Magnetic Resonance in Medicine, May 2022, Londres, United Kingdom. hal-04050550

HAL Id: hal-04050550

<https://hal.science/hal-04050550>

Submitted on 29 Mar 2023

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Jean-Lynce Gnanago¹, Tony Gerges¹, Laura Chastagnier², Emma Petiot², Vincent Semet¹, Philippe Lombard¹, Christophe Marquette², Michel Cabrera¹, Simon Lambert¹

¹Université de Lyon, INSA Lyon, Université Claude Bernard Lyon 1, Ecole Centrale de Lyon, CNRS, Ampère UMR5005, Villeurbanne, France.

²3d.FAB, Univ Lyon Université Lyon1 CNRS, INSA, CPE-Lyon ICBMS UMR 5246, Villeurbanne, France

3D MRI characterization of 3D printed tumor tissue models using a plastronic MR-Bioreactor : Preliminary results.

Synopsis

Tissue engineering for regenerative medicine have been developing for a few decades now and the number of applications is increasing to tackle the shortage of organ donors. To date, only few systems can allow both monitoring and 3D characterization of tissue constructs during their growth. In this study, we decided to focus on characterizing the Apparent Diffusion Coefficient (ADC) known to be a marker of cell density and built a MR-bioreactor to probe the ADC of a growing tissue. In this preliminary work, we were able to follow the cell density of our tissues.

Introduction

Tissue engineering for regenerative medicine have been developing for a few decades now and the number of applications is increasing to tackle the shortage of organ donors. From cartilage to skin, a wide range of tissues are currently being studied with the goal of restoring or replacing damaged tissues¹. While massive progress has been achieved to produce 3D printed constructs, only few systems can allow both monitoring and 3D characterization of tissue constructs during their growth^{2,3}. Despite a lower spatial resolution compared to optical modalities, Magnetic Resonance Imaging (MRI) allows non-destructive 3D characterizations of soft tissues based on multiscale parameters that are key in assessing tissue development^{4,5}. In this study, we decided to focus on characterizing the Apparent Diffusion Coefficient (ADC) known to be a marker of cell density⁶ and built a MR-bioreactor to probe the ADC of a growing tissue^{5,7}. Performing MR characterization of growing tissues is challenging because it requires to couple an imaging apparatus and a bioreactor which are usually not MR-compatible. To our knowledge, only one group tackled this issue by building a dedicated MR-compatible bioreactor³ to be used with a commercial surface MR coil. Generally, those coils are large (~1-10cm) compared to engineered tissues (~0.1-5cm) and thus do not allow optimal MRI conditions. In addition to that, using such a setup consisting in two devices adds complexity to an already complex modality, especially for non-experts. The resulting poor integration of the bioreactor and the MR coil can result in non-reproducible measurements. Hence, we decided to pursue our previous work² and propose here an improved MR-bioreactor for ADC assessment of a 3D printed tumor tissue model.

Method

Based on our previous work based on plastronic techniques^{2,8}, both the electrical and mechanical aspects of our MR-bioreactor were improved.

The coil geometry and the passive decoupling scheme remained unchanged but two electrical connections needed to be modified. First, the connection between the coil and the decoupling circuit integrated within the top cap has been made using a pair of twisted vias in order to avoid parasitic loops leading to imaging artifacts (figure 1a,1b). Second, the connection between the MR-bioreactor and the scanner has been displaced to an external 3D printed cover to reduce the mechanical load on the MR-bioreactor copper tracks (figure 1c). The connection between this cover and the MR-bioreactor is made using shield fingers (figure 5a).

Mechanical stability and hence measurement repeatability of the experiments were improved with a bench support designed specifically for our imaging platform (figure 5b,5c).

The imaging platform we used was equipped with a 7 T Bruker MRI Scanner running ParaVision 5.1 and a transmitting 72mm birdcage.

To illustrate the ability of our MR-bioreactor to obtain morphological images of small samples we 3D printed the logo of our laboratory and filled the MR-bioreactor with a 0.9% sodium chloride solution.

3D printed tissue models were provided by 3d.FAB platform. At time $t=0$, 3 tissues samples were printed and put inside a 37°C 5% CO₂ dedicated incubator. The tissues samples were 80% porous hydrogel scaffolds containing HT29 cells and fibroblasts (CAF). Each week, one sample was placed in our MR-Bioreactor for MRI characterization. After the MRI experiment, a calcein-am marking of living cells was performed on the sample.

During the experiments, tissues were immersed in a 0.9 % sodium chloride solution to ensure tissue viability during the acquisition time. Fluid circulation was also needed for removing air bubbles.

The samples were imaged using three acquisition sequences:

- Two morphological sequences. One axial and one coronal TurboRARE T2 (Repetition time (TR)=3700ms, Echo time (TE)=20ms, 16 averages, Field-of-view(FOV)=1.92*1.92, 256² matrix, 400 microns slice thickness)
- One quantitative EPI ADC diffusion sequence (TE=19.39ms, TR=5250ms, 8 segments, 8 averages, FOV=1.92*1.92, 128² matrix, $b=0\ 100\ 200\ 300\ 500\ 750\ 1000\ \text{s/mm}^2$) in order to quantify the ADC.

Regions of interest (ROI) were positioned within the sample. The ADC was computed using the ParaVision's post-processing tool.

Results

Figures 1a,1b demonstrate the artefact suppression using our twisted vias. Moreover, figures 1b,1c shows how having an additional connection with a connected cover between the MR-bioreactor and the terminal can be detrimental to the SNR.

A 40µm in plane resolution image of the logo of our laboratory can be seen figure 2.

A decreasing tendency of the ADC over the three weeks can be seen figure 3. On figure 5, the calcein AM marking of the living cells illustrate an increasing number of cells which is in agreement with the results shown by the ADC.

Conclusion

In this preliminary work, we were able to follow the cell density of our tissues. An analytical model⁹ along with a higher sampling rate would allow to derive the proliferation and cell motility from our

measurements. However, that would necessitate to have a more precise positioning of the sample with no displacement or deformation which is challenging due to samples' mechanical properties and fluid circulation. In any case, those results are the first step towards in vitro 3D tumor tissue model characterization.

Acknowledgement

This work was supported by a grant from the Agence National de la Recherche (Estimate Project N° ANR-18-CE19-0009-01). The financial support provided by Ingénierie@Lyon, member of the Carnot Institutes Network (Metafab 3D project) for the postdoctoral scholarship of Dr T. Gerges is also acknowledged. Moreover, the role of CERMEP - Imagerie du vivant and especially Radu Bolbos is acknowledged.

Figures

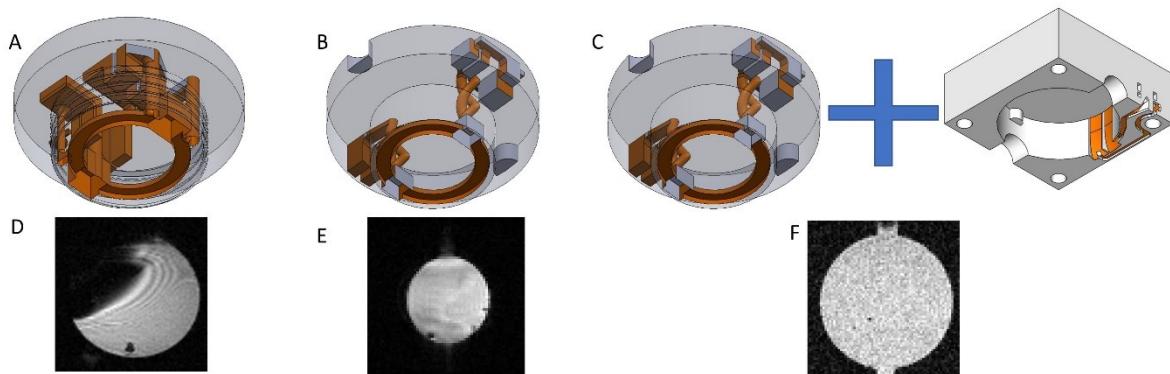


Figure 1: Electronic conception of the MR-bioreactor and its effects (A) Previous work exhibiting an imaging artefact. (B) Second version with twisted vias and a direct connection to the scanner (C) Third version with twisted vias and a connection to the scanner using an additional 3D printed housing. (D) 3D Flash ($150\mu\text{m}^3$ resolution $\text{TR}=33,81\text{ms}$, $\text{TE}=15\text{ms}$) image produced by the first prototype (E) 3D Flash ($300\mu\text{m}^3$ resolution $\text{TR}=33,81\text{ms}$, $\text{TE}=15\text{ms}$) image produced by the second prototype (F) 3D Flash image ($200\mu\text{m}^3$ resolution, $\text{TR}=15\text{ms}$, $\text{TE}=3\text{ms}$) produced by the third prototype.

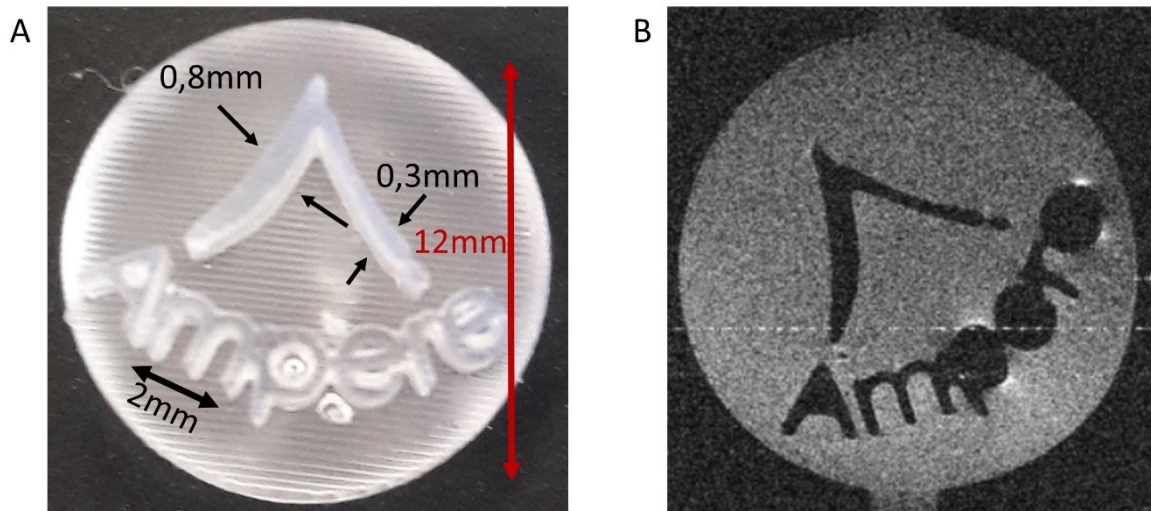


Figure 2: 3D Printed sample for our MR-bioreactor characterization. (A) A picture of the 3D printed sample made with the logo of our laboratory (B) The resulting turbore 2D ($40\mu\text{m}$ in plane resolution, slice thickness= $400\mu\text{m}$, TR= 2000ms , 8 avg) image using our MR-bioreactor.

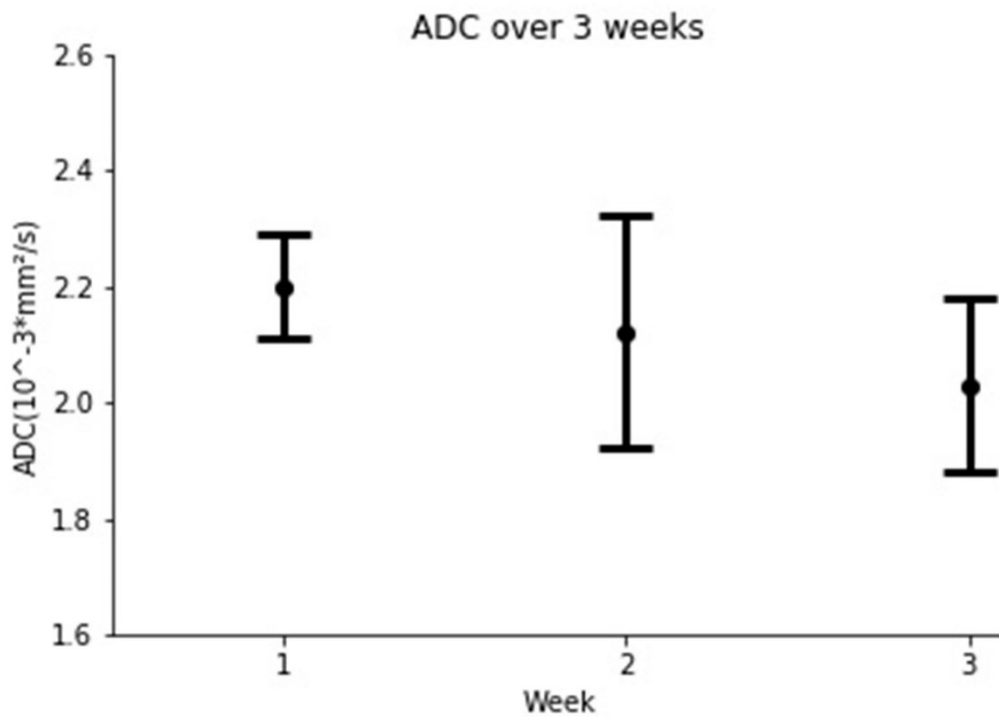


Figure 3: The evolution of the Apparent Diffusion Coefficient over the three weeks of experiments. The error bars are the standard deviation calculated with Paravision 5.1 post-processing tool.

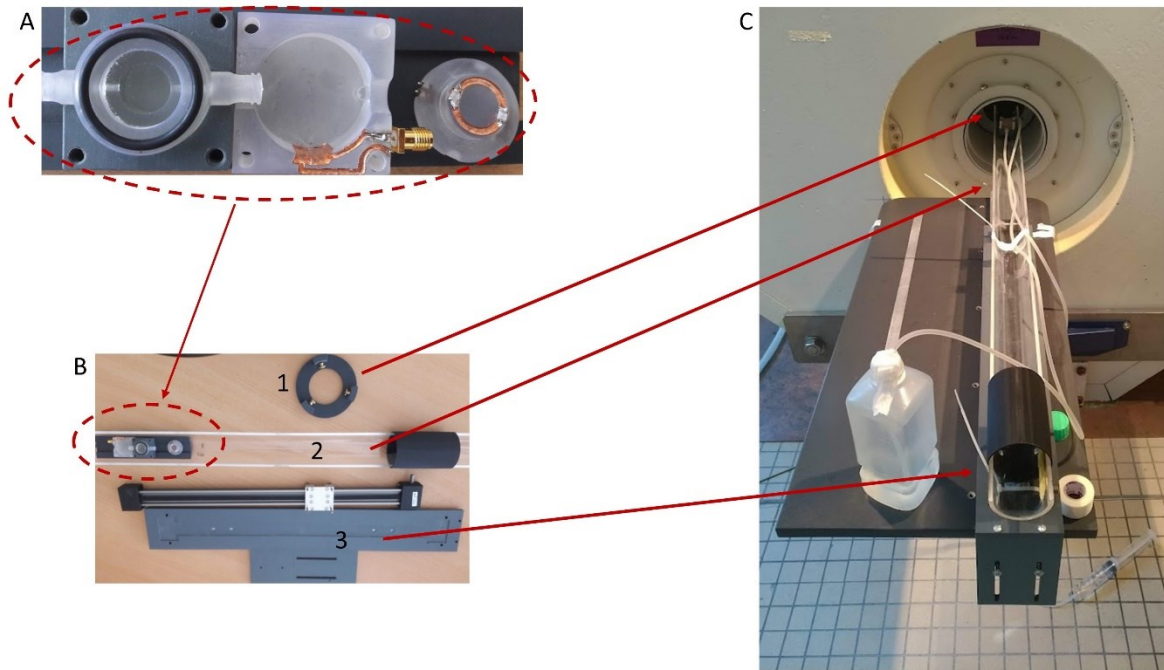


Figure 4: The MR-Bioreactor complete set-up. (A) A close-up of our MR-Bioreactor and its dedicated housing. (B) A view of the separated pieces of our MR-Bioreactor setup. 1. A centering ring designed to ensure the perfect alignment of the setup within the bore. 2. A PMMA bench carries the MR-bioreactor inside the bore. 3. A support designed to adapt the bench to the MRI platform. (C) A complete view of the set-up in place.

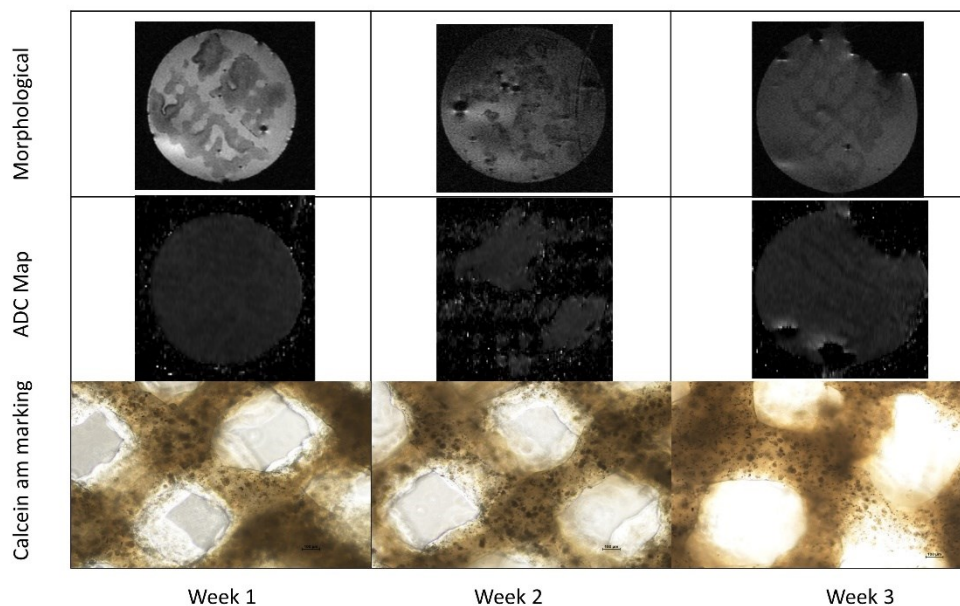


Figure 5: General view of the images acquired during the 3-week study using our MR-Bioreactor and a calcein am marking. Morphological images can be seen in the top row. The ADC maps can be seen in the middle row. The images of the living cells using a calcein am marking can be seen in the bottom row.

References

1. Langer, R. & Vacanti, J. Tissue engineering. *Science* **260**, 920–926 (1993).
 2. Gnanago, J.-L. *et al.* Compact MRI bioreactor for real-time monitoring 3D printed tissue-engineered constructs. in *Proceedings of the 29th International Society in Magnetic Resonance for Medicine Annual Meeting* 3122 (2021).
 3. Othman, S. F., Wartella, K., Khalilzad Sharghi, V. & Xu, H. The e-Incubator: A Magnetic Resonance Imaging-Compatible Mini Incubator. *Tissue Engineering Part C: Methods* **21**, 347–355 (2015).
 4. Frese, J. *et al.* Nondestructive monitoring of tissue-engineered constructs. *Biomedical Engineering / Biomedizinische Technik* **59**, (2014).
 5. *Magnetic resonance imaging in tissue engineering*. (Wiley Blackwell, 2017).
 6. Padhani, A. R. *et al.* Diffusion-Weighted Magnetic Resonance Imaging as a Cancer Biomarker: Consensus and Recommendations. *Neoplasia* **11**, 102–125 (2009).
 7. Kotecha, M., Schmid, T. M., Odintsov, B. & Magin, R. L. Reduction of water diffusion coefficient with increased engineered cartilage matrix growth observed using MRI. in *2014 36th Annual International Conference of the IEEE Engineering in Medicine and Biology Society* 3913–3916 (IEEE, 2014). doi:10.1109/EMBC.2014.6944479.
 8. Gerges, T. *et al.* 3D Plastronics for Smartly Integrated Magnetic Resonance Imaging Coils. *Front. Phys.* **8**, 240 (2020).
 9. Ellingson, B. M. *et al.* Spatially quantifying microscopic tumor invasion and proliferation using a voxel-wise solution to a glioma growth model and serial diffusion MRI: Image Maps of Tumor Invasion and Proliferation. *Magn. Reson. Med.* **65**, 1131–1143 (2011).
- 3d.Fab Platform <http://fabric-advanced-biology.univ-lyon1.fr/>