Decellularized Plant Scaffolds as 3R-Aligned Platforms for Building a Physiologically Relevant and Functional Three-Dimensional (3D) Human Intestine Model

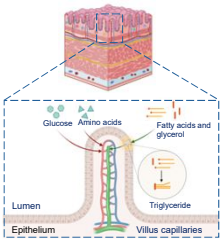
Didem P. Esmer [1], Hilal Özdemir [2,3], Merve Ünal [3], Şükrü Güleç [2], Serkan Dikici [1]

[1] Department of Bioengineering, Izmir Institute of Technology, Urla, Izmir, Turkey
[2] Molecular Nutrition and Human Physiology Laboratory, Department of Food Engineering, Izmir Institute of Technology, Urla, Izmir, Turkey
[3] Department of Biotechnology, Izmir Institute of Technology, Urla, Izmir, Turkey
didemesmer@iyte.edu.tr

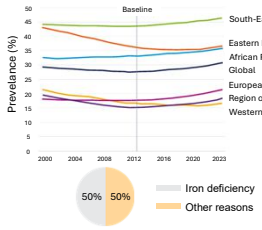
INTRODUCTION

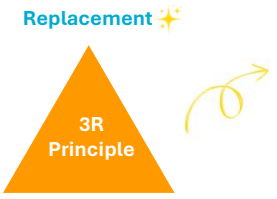
Aim: To develop plant-based, anatomically and physiologically relevant *in vitro* 3D human intestinal model on decellularized parsley stems and spinach leaves in line with 3R principle.

Small intestine epithelium



Prevelance of anemia among women aged 15 - 49 years

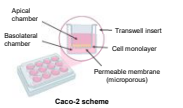




3R principle in animal research was first proposed by **William M.S. Russell** and **Rex L. Burch** in 1959.

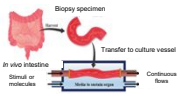
Current Existing Models and Their Properties

In vitro Models



- Originated from cell line
- Low simulation
- Low deviation
- Features
  - Easy to configure
  - Limited to simulate cell components and tissue

Ex vivo Models



- Harvesting tissue from animals
- Complete structure
- Large deviation
- Features
  - Relatively simple already formed and available
  - Hard to maintain

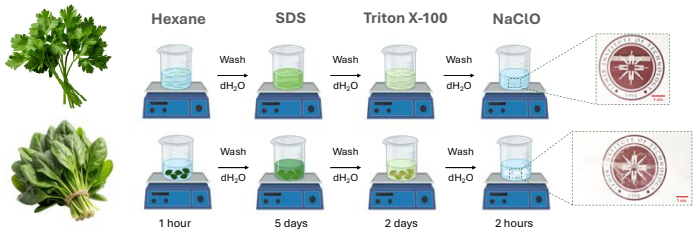
In vivo Models



- A complete model of body
- Large deviations among animal models
- Features
  - Best tissue representation
  - Complex procedures
  - Give rise to ethical concerns

METHODOLOGY

1 - Decellularization of Parsley Stems and Spinach Leaves



2 – Morphological, Chemical, Mechanical, and Physical Characterizations

Scanning Electron Microscopy (SEM)



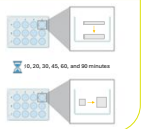
Fourier Transform Infrared Spectroscopy (FTIR)



Tensile Test

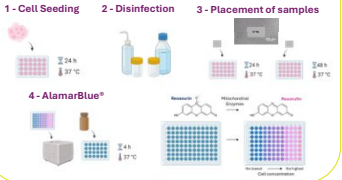


Swelling

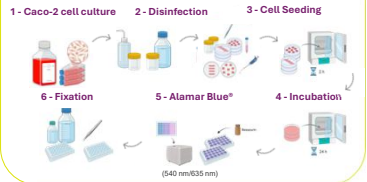


3 - Biological Characterizations

Cytotoxicity assessment with L929 cells based on ISO 10993-5 (Annex C)



Development of *in vitro* human intestine model using Caco-2 cells



4 - Evaluation of the Functionality of in vitro Intestine Model By Iron Deficiency Anemia (IDA) Model

Inducing Iron Deficiency Anemia Model



Functionality

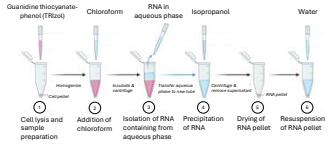
- Monolayer formation
- Tight Junction Proteins *(ZO-1, OCLN)*
- Alkaline Phosphatase Activity (ALP)

Physiological Relevance

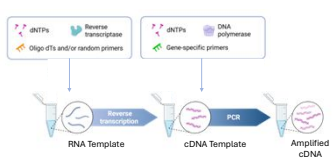
- Development of DFO induced IDA model *(DMT1, TFR, ANKRD37 mRNA expression levels)*↑
- Treatment of IDA model with FAC *(DMT1, TFR, ANKRD37 mRNA expression levels)*↓

Gene Expression Analysis by RT-qPCR

1- mRNA Isolation

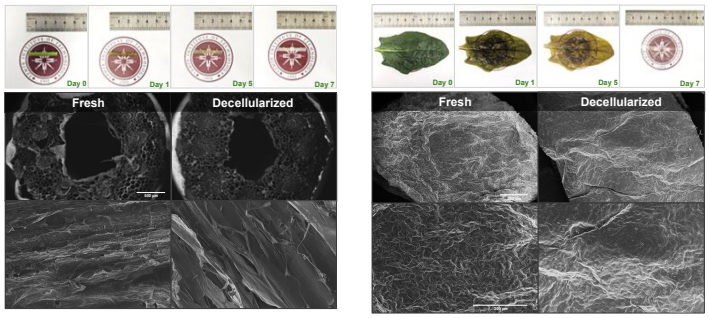


2 - cDNA Synthesis and Real-Time Polymerase Chain Reaction (RT-qPCR) Analysis



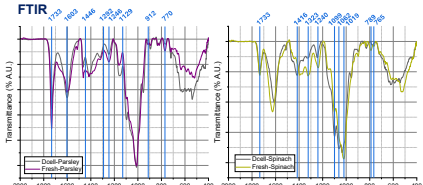
RESULTS

1 - Plant Decellularization and Morphological Characterization



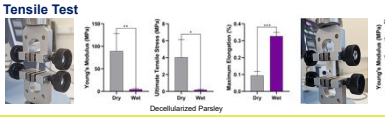
2 - Chemical, Mechanical, and Physical Characterizations

FTIR

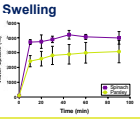


Wavenumbers (cm⁻¹)	Functional Group Assignment	Structural Interpretation
1733	C=O stretching (lipids)	Elimination of lipid-related structures
1603	Asymmetric carboxylate and C=C ring stretching of phenolic compound	Alterations in the relative pectin content
1446	CH2 vibration (cellulose and aliphatic compounds in the cuticle)	Removal of cuticles from the leaf
1323	Crystalline structure of the cellulose	Structural shift from cellulose I to cellulose II
1416	Amide III (C-N stretching and N-H bending)	Associated with removal of protein related structures
1292	Symmetric P-O stretching vibration in the DNA/RNA backbones	Removal of phosphorylated proteins and polyphosphate storage products
1246	C-O and C–O–C stretching of cellulose and non-cellulosic materials such as hemicellulose and pectin	Retention of structural materials, including cellulose, hemicellulose, and pectin
1240	β linkage and C-O-C stretching of cellulose	Confirmation of the preservation of the cellulose
1129	Sugar-phosphate vibration in DNA structure	Removal of DNA
1062		
1099		
1019		
912		
789		
770		
765		

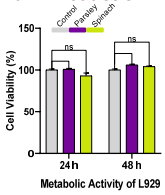
Tensile Test



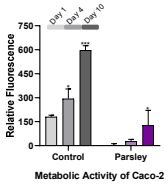
Swelling

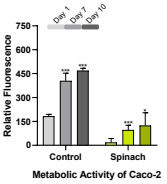


3 - Cytotoxicity Assessment and Metabolic Activity Measurement of Caco-2 cells by Resazurin Reduction Assay (AlamarBlue®)

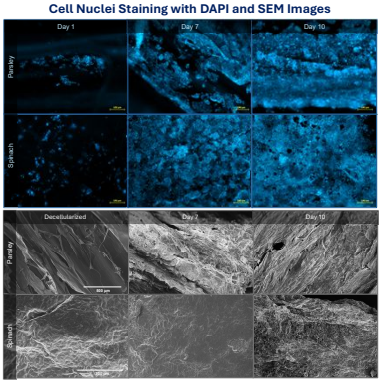


Cell viability > **70%** no cytotoxic effect



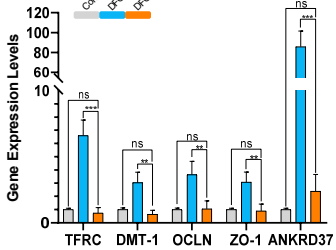


Cell Nuclei Staining with DAPI and SEM Images



4 - Quantification of mRNA Expression by RT-qPCR

Functional Assessment of Intestinal Model on Decellularized Spinach



CONCLUSION & FUTURE WORKS

- Fabrication of decellularized parsley stems and spinach leaves
- Evaluation of morphological, chemical, physical, and mechanical properties
- Assessment of the cytotoxicity of the developed biomaterials based on ISO 10993-5 (Annex C) guidelines
- Development and characterization of an *in vitro* human intestine model using Caco-2 cells
- Evaluation of the physiological relevance and functionality of the developed intestinal model using an iron-deficiency anemia model, and determination of cell differentiation by ALP.

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4. Temraz, S., Santini, V., Musallam, K. & Taher, A. Iron overload and chelation therapy in myelodysplastic syndromes. Critical Reviews in Oncology/Hematology vol. 91 64–73 (2014).
5. Steinbicker, A. U. & Muckenthaler, M. U. Out of balance-systemic iron homeostasis in iron-related disorders. Nutrients vol. 5 3034–3061 (2013).

ACKNOWLEDGEMENTS

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