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Supplementary Data

Characterization of Innately Decellularised Micropattern Pseudostem of *Musa balbisiana* – A Non-surface Functionalized 3D Economic Biomaterial Scaffold

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1. Supplementary Methods

1.1. Sourcing of the MPM-3Ds material, preservation, sterilization and 3D scaffold incorporated polystyrene plate development

The supportive outermost pseudostem sheath of the banana plant selected for preparing the 3D scaffold consists of tightly packed overlapping sheaths of leaves. The scaffold material retrieved from the live plant by excising the outer sheaths trimmed using a sharp scalpel blade exposes the micropatterned niche [1-3]. The fresh-cut washed scaffolds were tailored and placed into 6 and 24 well polystyrene cell culture plates, frozen at -80°C and freeze dried (Neo cool Yamato) for a 48-h cycle. MPM-3Ds embedded well plates were then wrapped in specific indicator packs and sterilized by passing ethylene oxide gas (ETO) at the Microtrol Sterilisation Services Pvt. Ltd. Kochi to emerge as a ready to use, economical 3D cell culture plate. Detection of fungal spores and bacteria using a microbiological plating ensured ETO sterilization effectiveness.38g/L Muller-Hinton Agar (Hi media M173) and 39g/L Potato Dextrose Agar (Hi media M096) were added separately to distilled water, boiled, and then sterilized by autoclaving at 15lbs pressure (121°C) for 15 minutes. The agar poured into sterile culture dishes, and once set, the fresh, freeze-dried, and ETO sterilized MPM-3Ds were placed on the respective agar and incubated at 37 °C for bacterial test and 26°C for fungal test for 24 hours and observed for contaminants.

1.2. Taxonomic identification of the MPM-3D source plant

DNA barcoding is a method for identifying the taxonomy of MPM-3Ds source banana plant [4, 5]. The primers chosen for the amplification of genes in the plant sample are Maturase K (matK), a chloroplast gene, ribulose bisphosphate carboxylase gene (rbcL), and transporter (Pho87 family) Photosystem II CP43 reaction center protein gene (trnS psbC) [6-8]. The DNA extracted from the mother plant and the matK, rbcL, and the trnS psbC genes were amplified and sequenced [9, 10]. The genes sequence was then compared to the NCBI database using the BLAST algorithm and matched.

Bioversity International manages Promusa and its website (www.promusa.org) as part of the Consultative Group for International agriculture research (CGIAR), thereby is accepted as an authentic source. To determine the plant's taxonomy, the morphology of the different parts of the source banana plant, its inflorescence, and the fruits photographed (HTC), compared to the banana plant identification data provided in http://www.promusa.org taxonomical identification [11, 12].

1.3. MPM-3Ds Characterisation

1.3.1. Detection of the functional groups present on MPM-3Ds

Fourier transform infrared spectroscopy (FT-IR) was used to identify the different functional moieties of the scaffolds. The MPM-3Ds were analyzed using a Thermo Nicolet Avatar 370 spectrometer against a blank KBr pellet background at wave number 4000-400 cm^{-1} and spectral resolution of 4 cm^{-1} [7].

1.3.2. Evaluation of the thermal stability of the MPM-3Ds

The thermal stability of the MPM-3Ds evaluated using Perkin Elmer Diamond thermal Analyser is used for material characterization through decomposition range and degradation profile analysis [13, 14].

1.3.3. Contact angle measurement of the MPM-3Ds

The hydrophilicity of the freeze dried MPM-3Ds measured by dropping deionized water automatically onto the flat surface of the scaffold using a video contact angle instrument (Rame Hart). The instrument's software displayed the contact angles measured [15, 16].

1.4. Biocompatibility, cell attachment and proliferation

1.4.1. Human pancreatic cancer cell line and Mouse fibroblast cell lines

MIA PaCa-2 (Human Pancreatic cancer cell lines) and L929 cells (Mouse fibroblast cell line) were purchased from NCCS Pune and maintained in Dulbecco's Modified Eagles medium (DMEM) supplemented with 10% FBS The cells incubated in a 5% CO2 incubator at 37 °C are detached from the flask with Trypsin-EDTA after attaining confluence. $2x10^5$ cells of MIA PaCa-2 and L929 were seeded on the respective media drenched freeze-dried and sterilized MPM-3Ds placed in well plates. The cell line seeded scaffolds were evaluated for cell attachment and proliferation by SEM.

1.5. *Scaffold processing for imaging*

The MPM-3Ds were retrieved after the experiments and visualized using a bright field microscope and fluorescent microscope for cell viability using Calcein AM (Invitrogen 20 X 50 UG Cat#C3100MP) adhering to the kit protocol. The scaffolds were processed for scanning electron microscopic studies to study the morphology of the cells attached to the MPM-3Ds. The medium was aspirated from the well, and the scaffolds gently washed twice with PBS solution to this 2% glutaraldehyde solution was added and incubated for 2h. The glutaraldehyde solution was aspirated from the scaffold and washed 3-4 times with PBS solution. The MPM-3Ds were dehydrated with ethanol (70%, 80%, 90%, and 100%) and observed under SEM.

2. Supplementary Results

2.1. Sourcing of the MPM-3Ds material, preservation, sterilization and 3D scaffold incorporated polystyrene plate development

The tailored banana pseudostem scaffolds placed in the six-well polystyrene cell culture plates were thoroughly freeze-dried and ETO sterilized. The dorsal and ventral side of the freeze-dried as well as fresh MPM-3Ds when cultured on a nutrient agar plate showed presence of bacterial growth (**Suppl. Figure 1-A**). However, similar experiments on ETO sterilized replicates showed no bacterial growth (**Suppl. Figure 1-B**). Further analysis of fungal growth in similar way showed similar pattern of fungal growth for non-sterilized (**Suppl. Figure 2-C**) *vs* no fungal growth upon post- ETO sterilization (**Suppl. Figure 1-D**). The ready to use MPM-3Ds incorporated cell culture well plate was successfully designed and sterilized, as shown in **Suppl. Figure 1-E**.

2.2. Taxonomic identification of the MPM-3D source plant

The morphological features of the banana plant's pseudostem and reproductive body, matched the *balbisiana* species as authenticated by Promusa (**Suppl. Figure 2**). The amplified gene sequence, was 96 percent similar to the Musa balbisiana as projected by the BLAST software of NCBI (**Suppl. Figure 3**). The evidence authenticates the genus

name as *Musa* and the source plant species as *balbisiana*, and hence, the scientific name of the pseudostem source plant was determined and confirmed as *Musa balbisiana*.

2.3. MPM-3Ds Characterization

2.3.1. Detection of the functional groups present on MPM-3Ds

FTIR spectral analysis shows the % transmittance peak of MPM-3Ds at 3433.44 cm^{-1} corresponds to the OH stretching vibration of water and–OH group. The peaks at 2050 cm^{-1} indicate existence of CH_2 vibration (**Suppl. Figure 4**). The peaks around 1630 cm^{-1} can be attributed to stretching of carbonyl group of lignin and aromatic ring skeleton vibration, respectively, and peak at 679cm^{-1} represents P-O stretching vibration. The IR of the functional groups detected signifies the presence of cellulose, pectin, and lignin in the scaffold [17].

2.3.2. Evaluation of the thermal stability of the MPM-3Ds

The thermo gravimetric graph, depicts %weight change of MPM-3Ds upon increase in temperature from 40 to 740 °C at a rate of 20°C /min. The material is stable at physiological temperatures, and the graph goes downhill around 100°C due to weight loss by moisture removal from scaffold. It can be observed that scaffold is stable up to 200°C. A sharp decrease in weight starts around 280 °C and extends up to 360 °C, further weight of the sample reduces to 20%. (**Suppl. Figure 5**)

2.3.3. Contact angle measurement of the MPM-3Ds

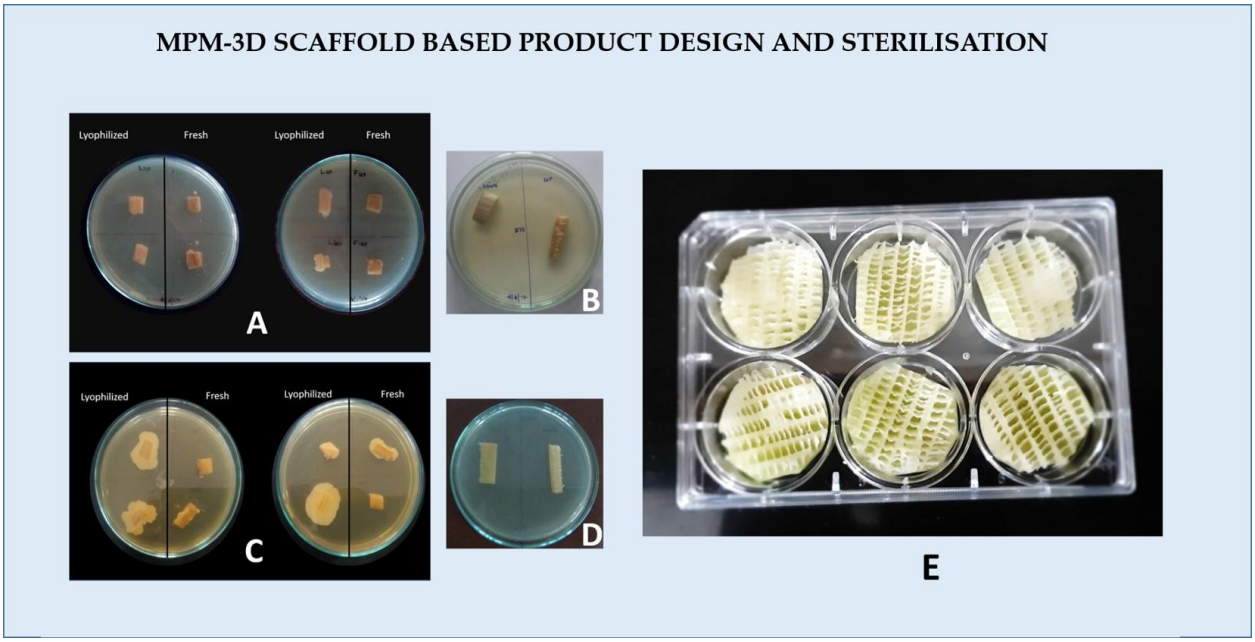
The freeze-dried scaffold shows an initial θ value of 53-55°. Once the initial drop got imbibed into dry MPM-3Ds, it absorbed next series of water droplets quickly, thereby shifting the θ value from 55° to 33°.

2.4. Biocompatibility, cell attachment and proliferation

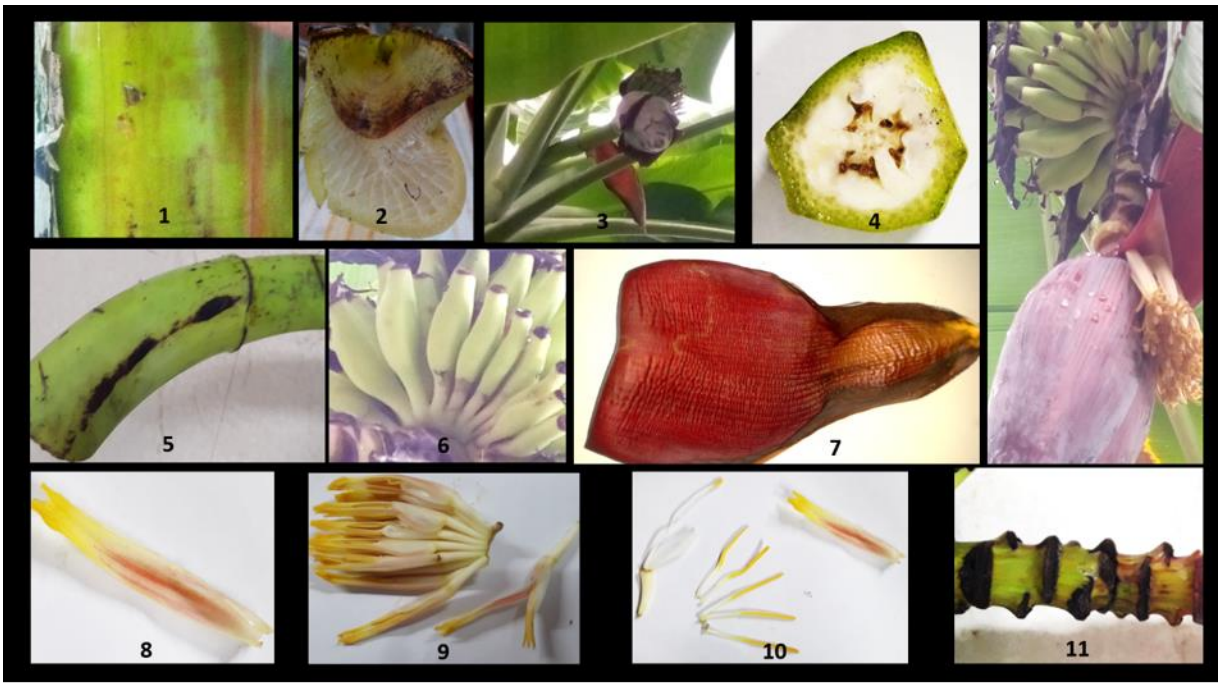
2.4.1. Human pancreatic cancer cell line and Mouse fibroblast cell lines

L929 cells, as seen in SEM image attached to the scaffold 2h, 24 h, and 14 days post-seeding (**Suppl. Figure 6**). The cells continued to proliferate on the scaffold for 14 days, which was kept as an endpoint for observation.

MIA PaCa-2 cells, as seen in the SEM image (**Suppl. Figure 7-C, D**), attached to the scaffold 24 h post-seeding. The cells continued to proliferate on the scaffold for 14 days, which was kept as an endpoint for observation (**Suppl. Figure 7-A, B**).

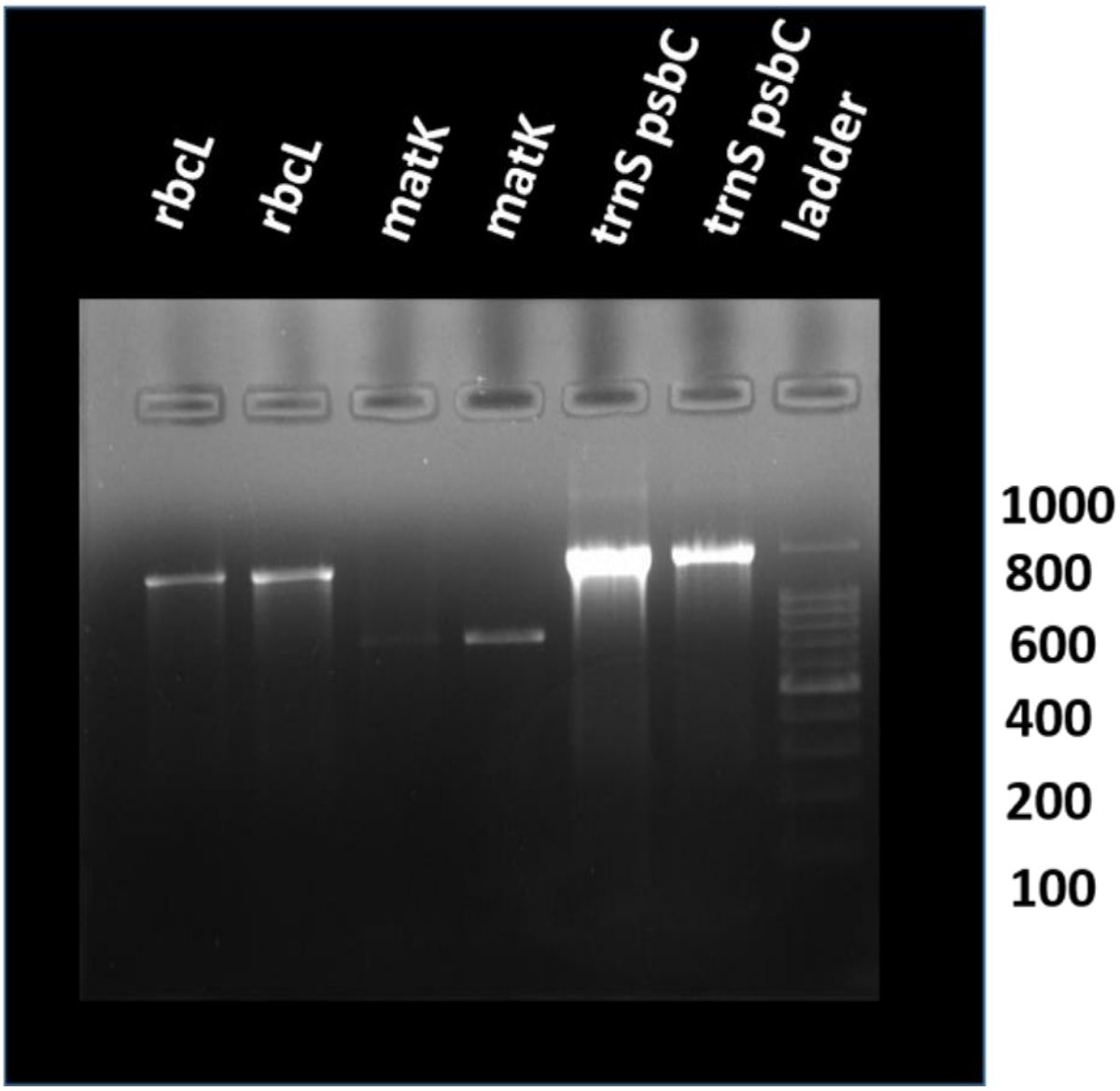


Suppl. Figure 1: (A, C) shows the bacterial and fungal load respectively in the freezedried and the fresh banana pseudostem scaffold; (B, D) Banana scaffold after ETO sterilization is respectively free of bacteria and fungus; (E) Photograph of MPM-3Ds incorporated sterile ready to use six-well cell culture plate.

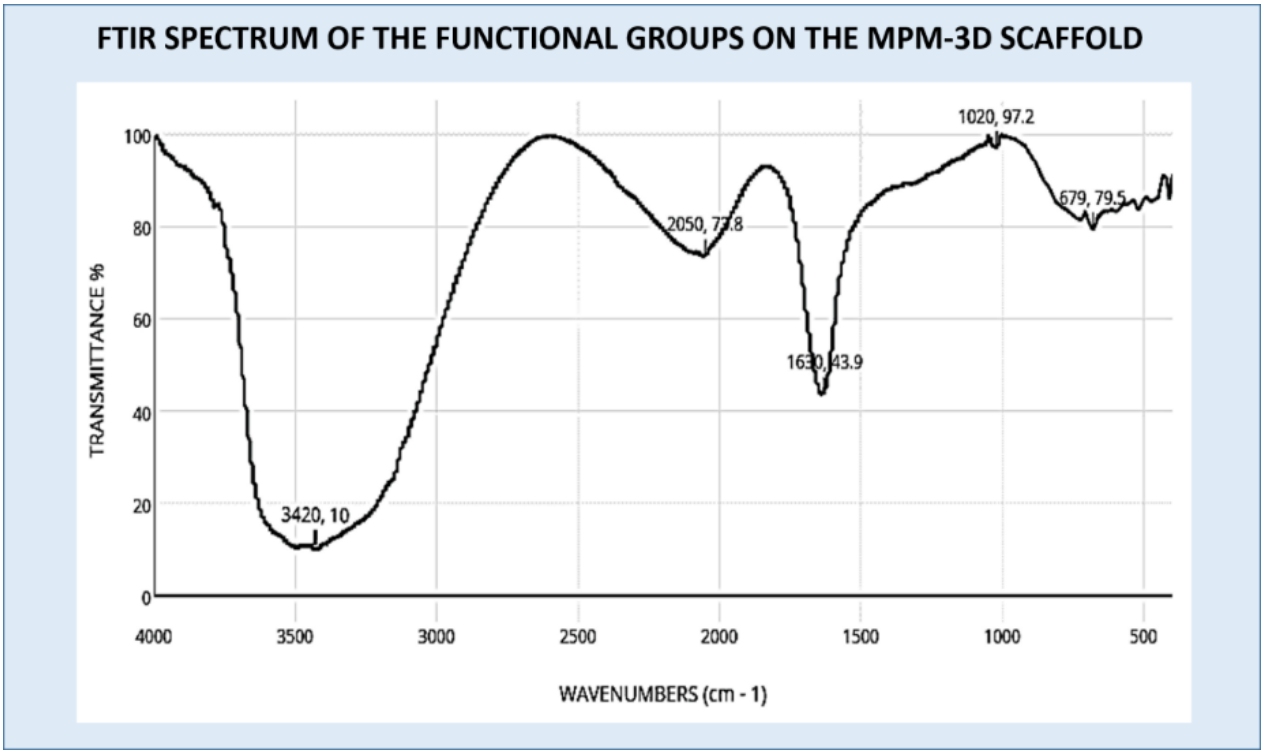


S.NO	SPECIFIC PARTS	CHARACTERISTICS
1	Pseudostem colour	More or less heavily marked with brown or black spots
2	Petiole canal	Margin erect or spreading , with scarious wings below, not clasping pseudo stem
3	Bract curling	Bract reflex and curl backward
4	Ovules	Three irregular rows
5	Peduncle	Smooth without hair- Glabrous
6	Pedicles	Long Pedicels
7	Bract	Shape- Broad ovulate not tapering sharply Apex- Obtuse Colour- Brownish purple outside and bright crimson inside continuous to the base
8	Free tepal of the male flower	Corrugated
9	Male flower colour	Flushed with pink
10	Stigma colour	Yellow
11	Bract scar	Prominent

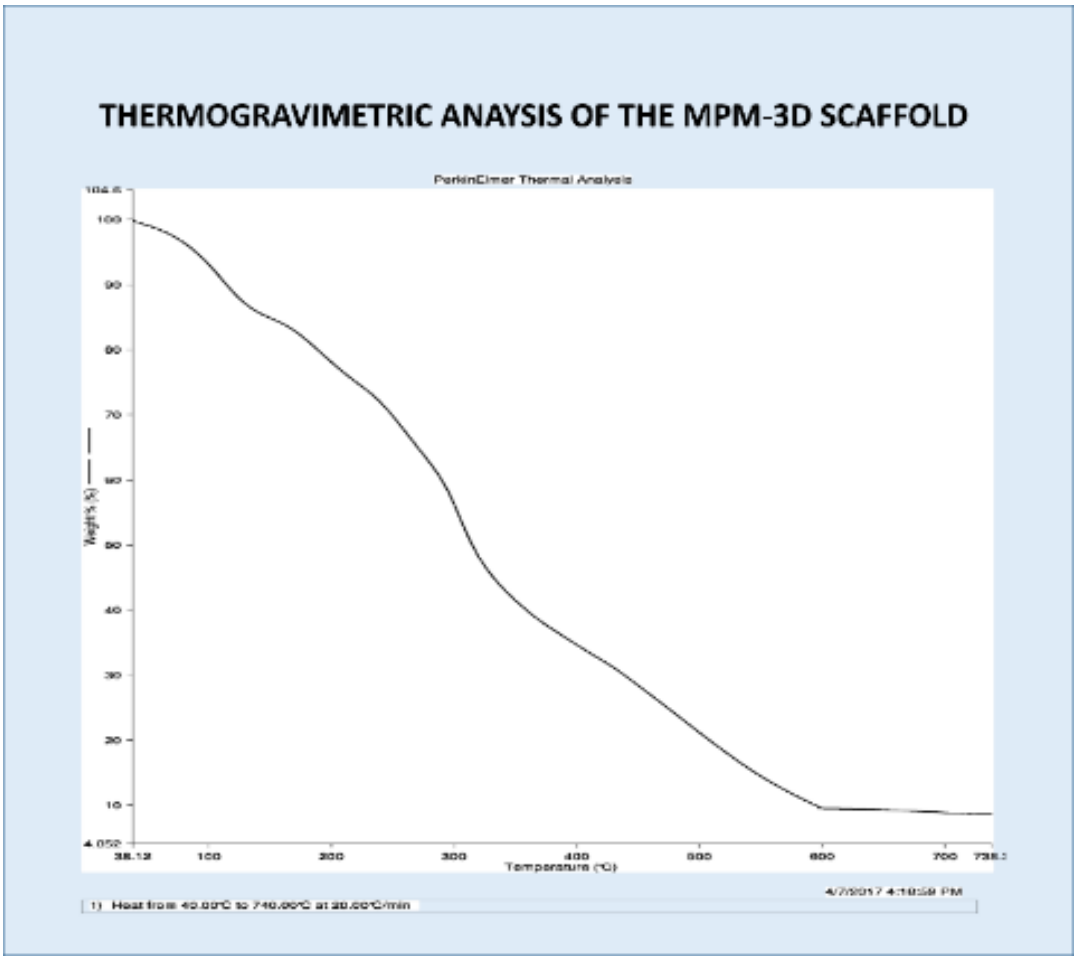
Suppl. Figure 2 Photograph and the corresponding morphological description of banana plant species identification (www.promusa.org)



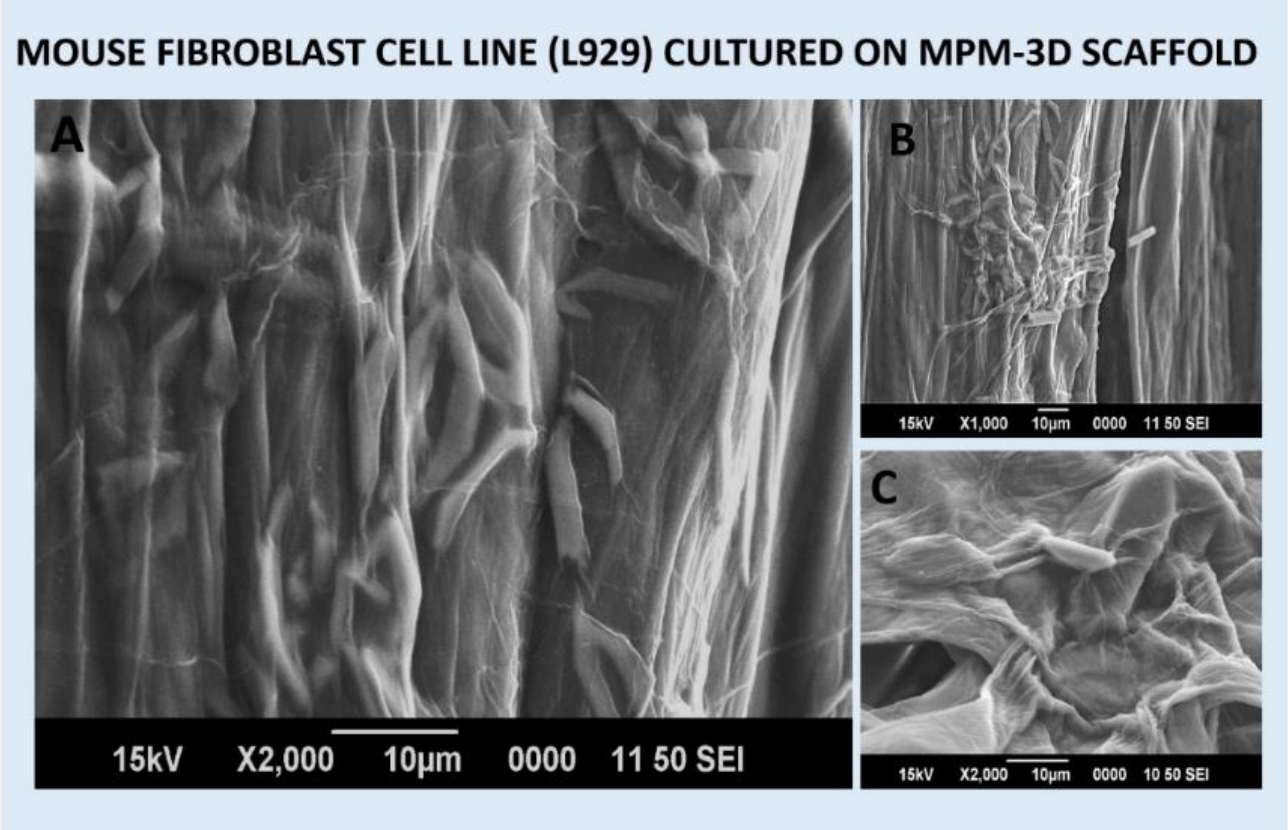
Suppl. Figure 3 Image shows the amplified genes for identifying the taxonomy of MPM-3Ds source banana plant by DNA barcoding.



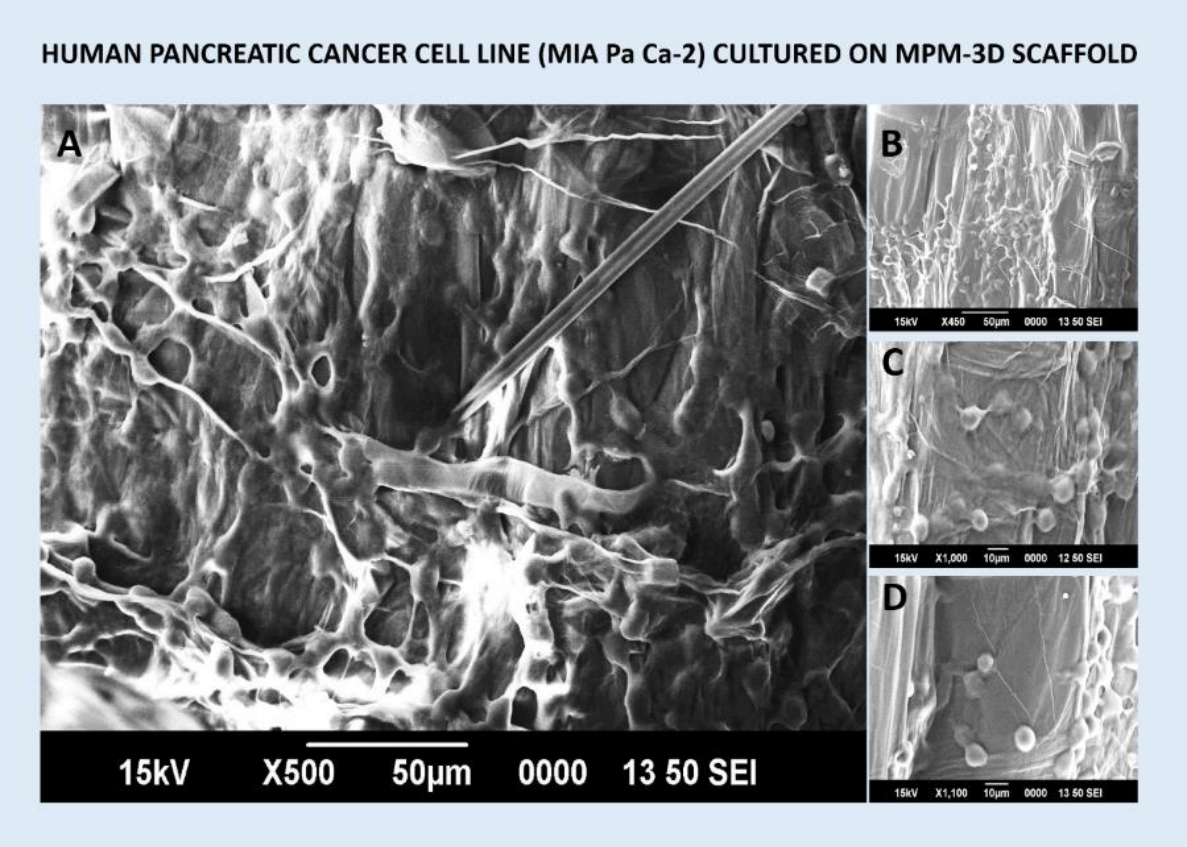
Suppl. Figure 4 FTIR spectrum of the functional groups present in the MPM -3D scaffold.



Suppl. Figure 5 The thermo gravimetric graph depicts %weight change of the MPM-3Ds concerning an increase in temperature from 40 to 740 °C at a rate of 20°C /min.



Suppl. Figure 6 SEM images of the L929 cells cultured on the MPM-3Ds .A-Cells at 2hrs, B-Cells at 24h and C cells at Day 14.



Suppl. Figure 7 SEM images of MIA Pa Ca-2 cells on the MPM-3Ds. A and B shows the cells at day 14 and C and D show cells at 24 h.

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