Journal Club Discussion:

ARTICLE

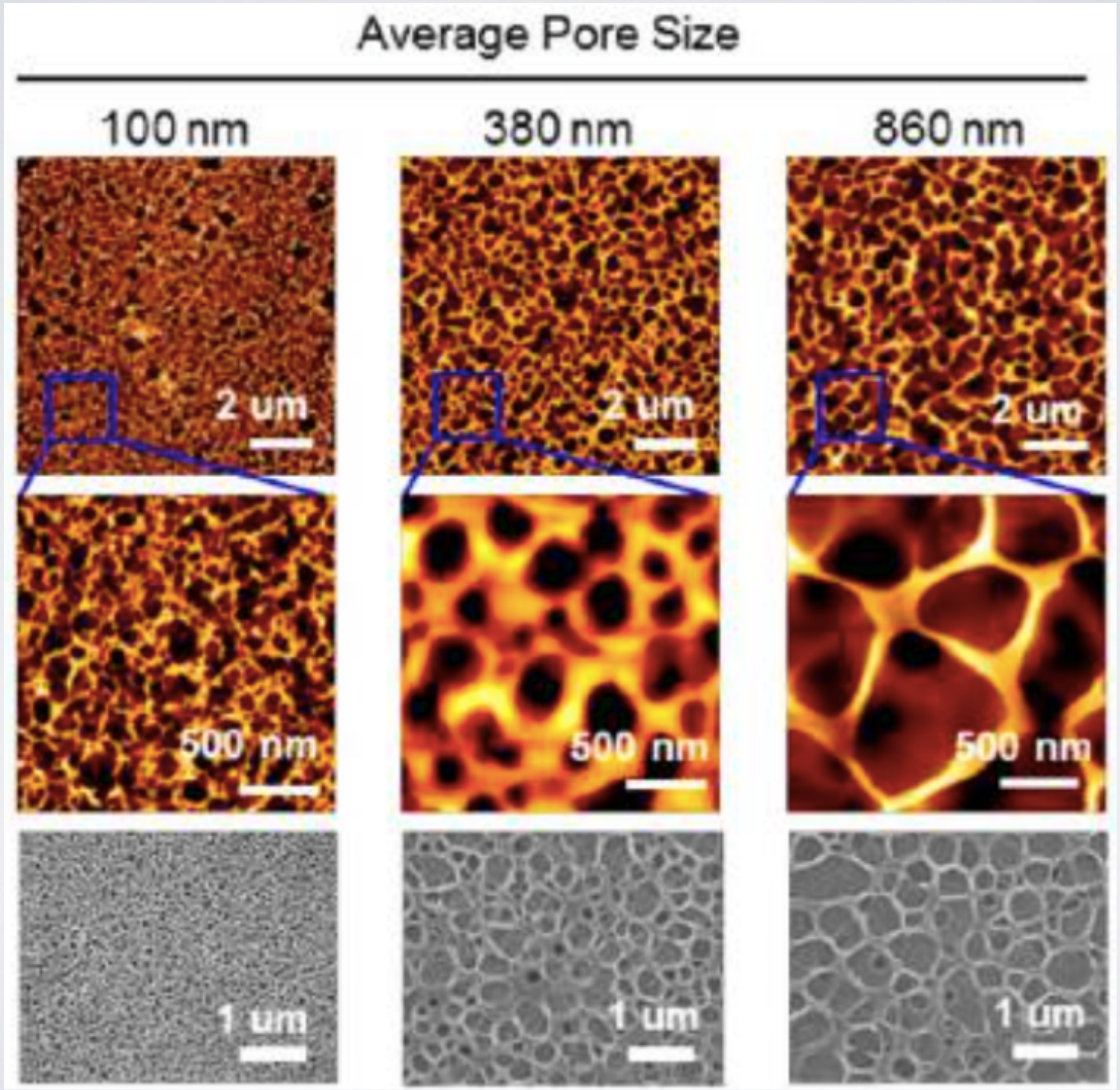
Nanothin Coculture Membranes with Tunable Pore Architecture and Thermoresponsive Functionality for Transfer-Printable Stem Cell-Derived Cardiac Sheets

Seungmi Ryu,[†,#] Jin Yoo,[‡,#] Yeongseon Jang,[‡] Jin Han,[§] Seung Jung Yu,[∥] Jooyeon Park,[§] Seon Yeop Jung,[⊥] Kyung Hyun Ahn,[§,⊥] Sung Gap Im,[∥] Kookheon Char,[*,‡] and Byung-Soo Kim[*,†,§,⊥]

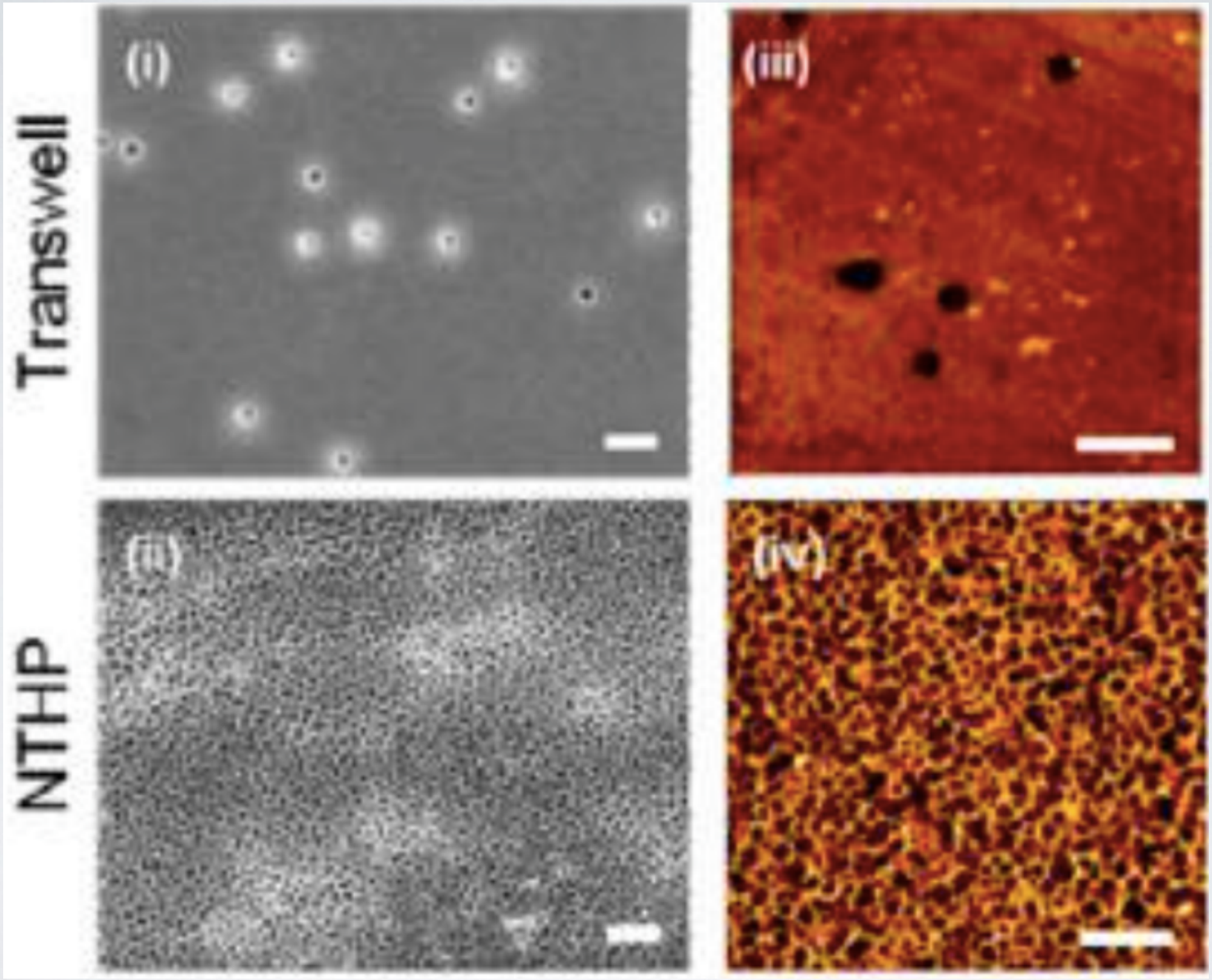
[†]Interdisciplinary Program for Bioengineering, [‡]World Class University Program for Chemical Convergence for Energy and Environment, The National Creative Research Initiative Center for Intelligent Hybrids, [§]School of Chemical and Biological Engineering, and [⊥]Institute of Chemical Process, Seoul National University, Seoul 151-744, Republic of Korea and [∥]Department of Chemical and Biomolecular Engineering & KI for Nano Century, Korea Advanced Institute of Science and Technology, Daejeon 305-701, Republic of Korea. [#]These authors contributed equally.

Oct/06/2015

NTHP (nanothin and highly porous) membrane

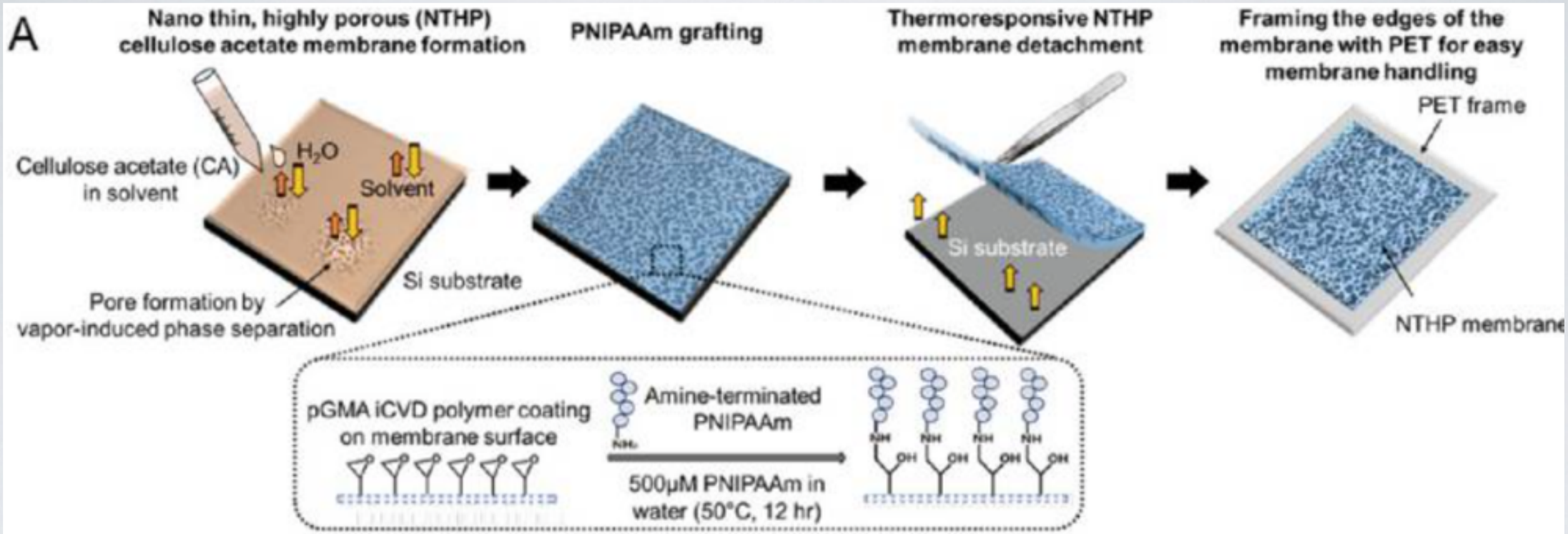


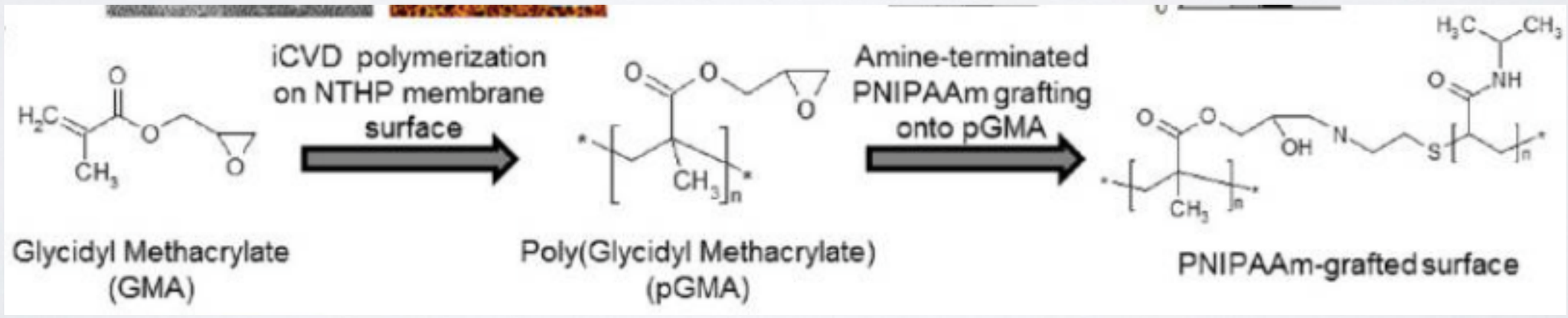
comparison to Transwell: porosity



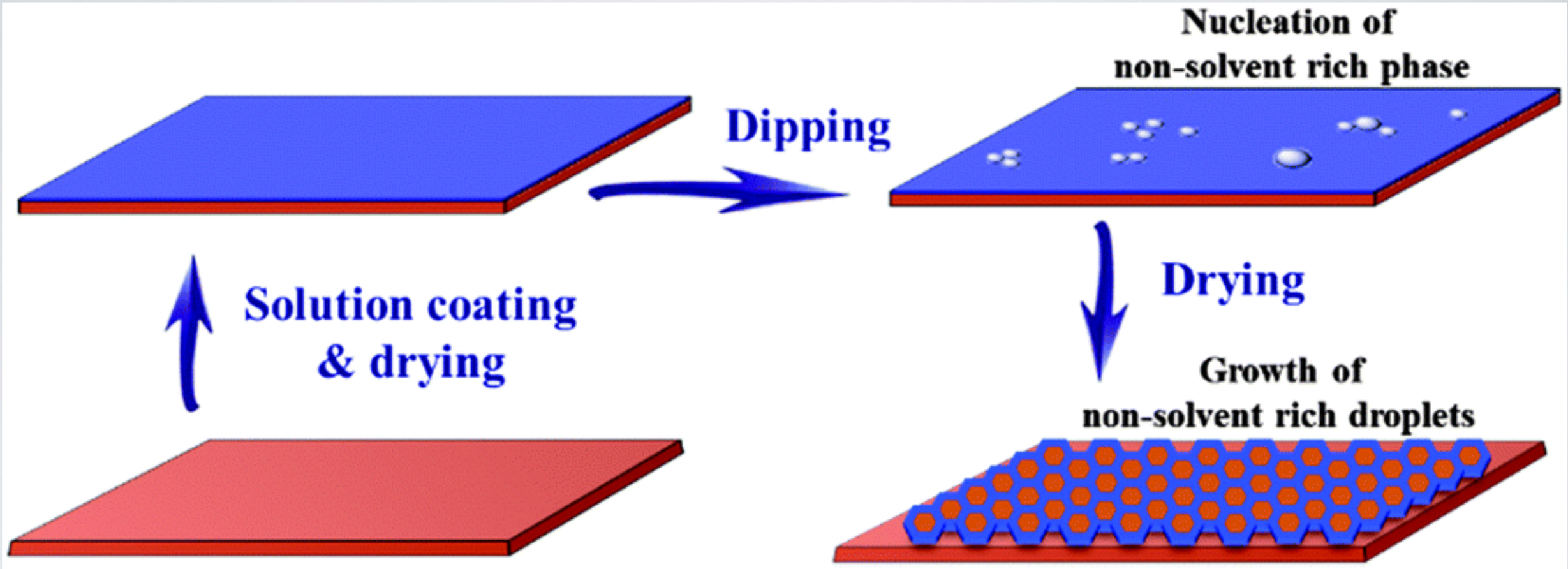
porosity of 2% (transwell) vs 54% (NTHP)

fabrication workflow of the NTHP membrane





VIPS (vapor-induced phase separation)

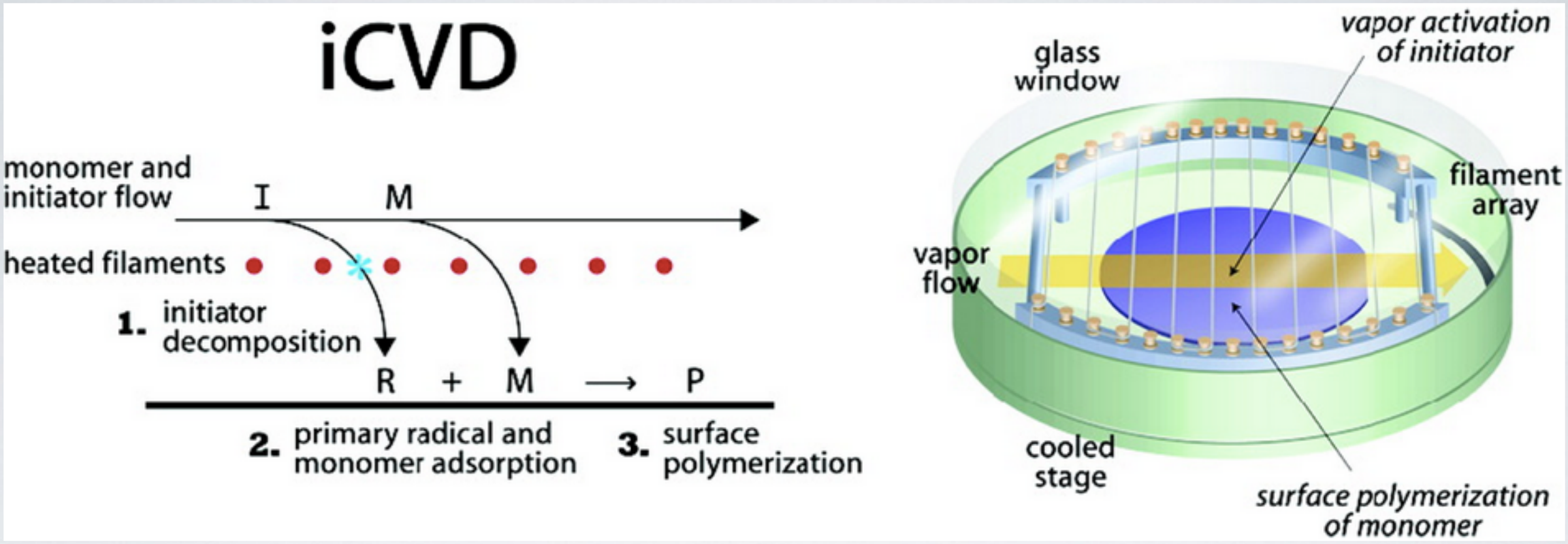


"bubbling" non-solvent into solvent to create "pores"

VIPS is the technique used to create the cellulose acetate (CA) membrane

NTHP-100: CA in acetone (3 wt%), spin coated at 3000 rpm for 25 sec
NTHP-380: CA in THF (4 wt%), spin coated at 3000 rpm for 25 sec
NTHP-860: CA in THF (3 wt%), spin coated at 1000 rpm for 25 sec

iCVD (initiator Chemical Vapor Deposition)

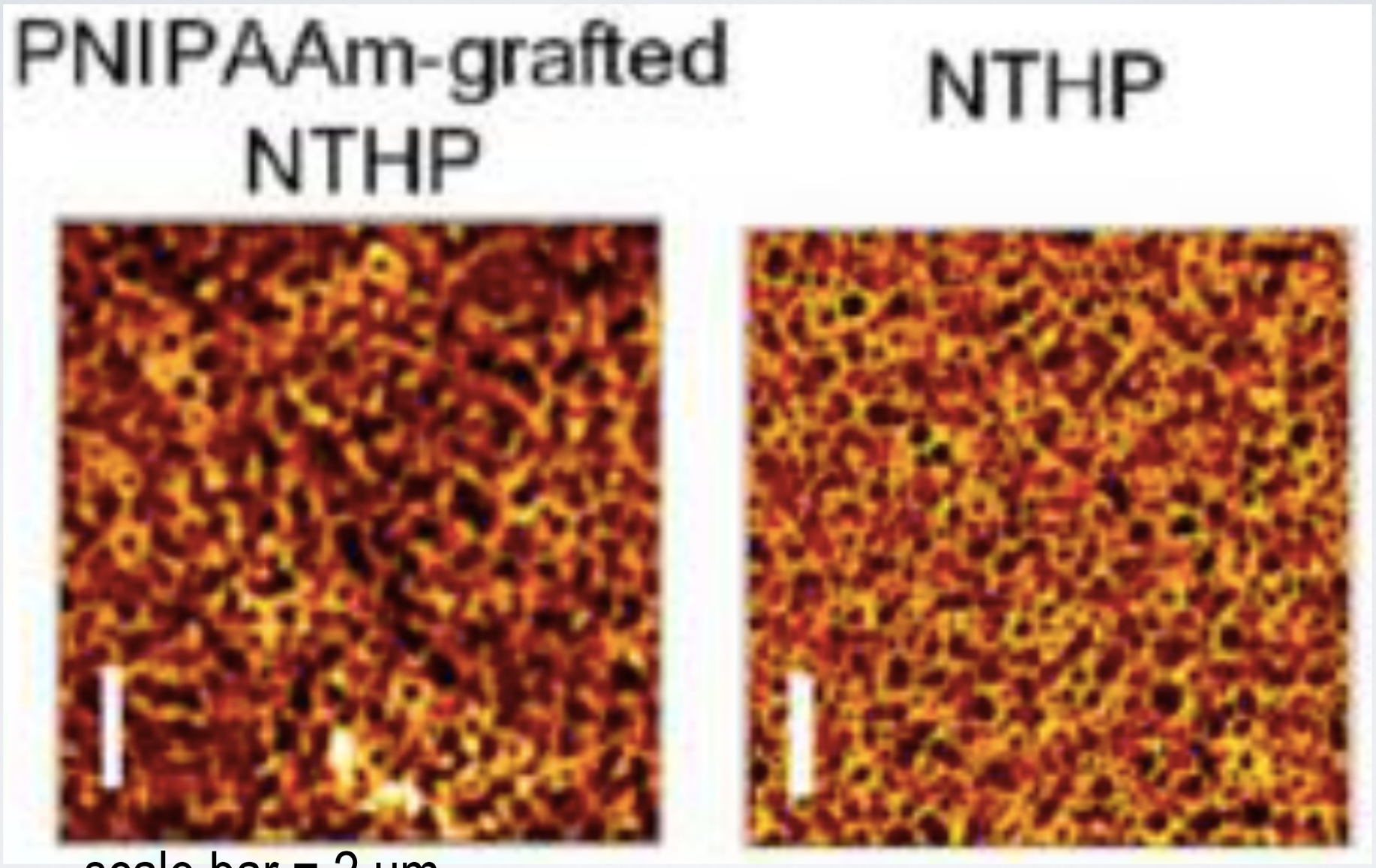


essentially, replicating solution phase free-radical polymerization in the vapor phase

initiator: TBPO (ter butyl peroxide)
monomer: GMA (glycidyl methacrylate)

amine-terminated PNiPAAm was then grafted onto the iCVDed sample via incubation

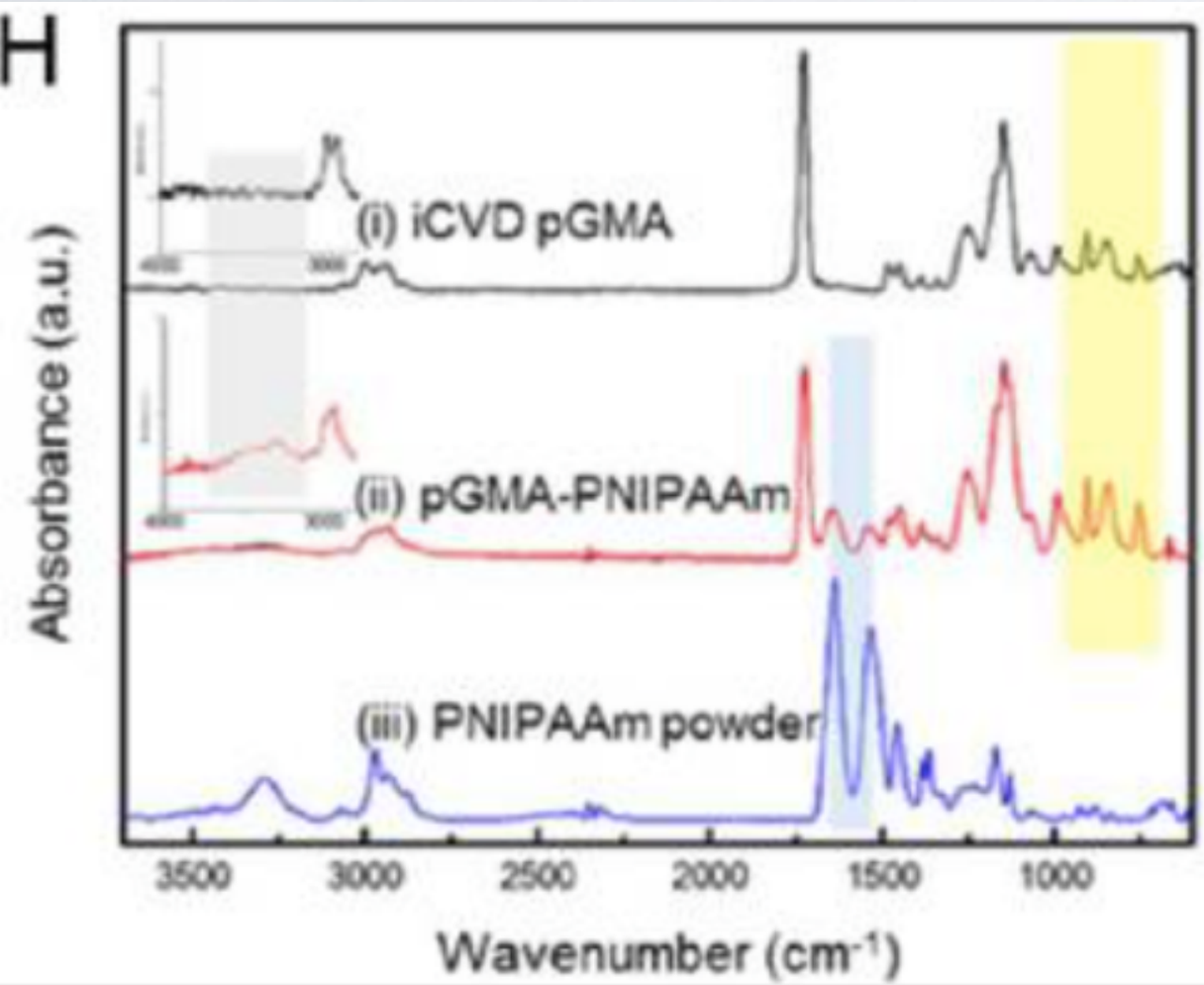
NTHP before and after PNiPMMAm grafting

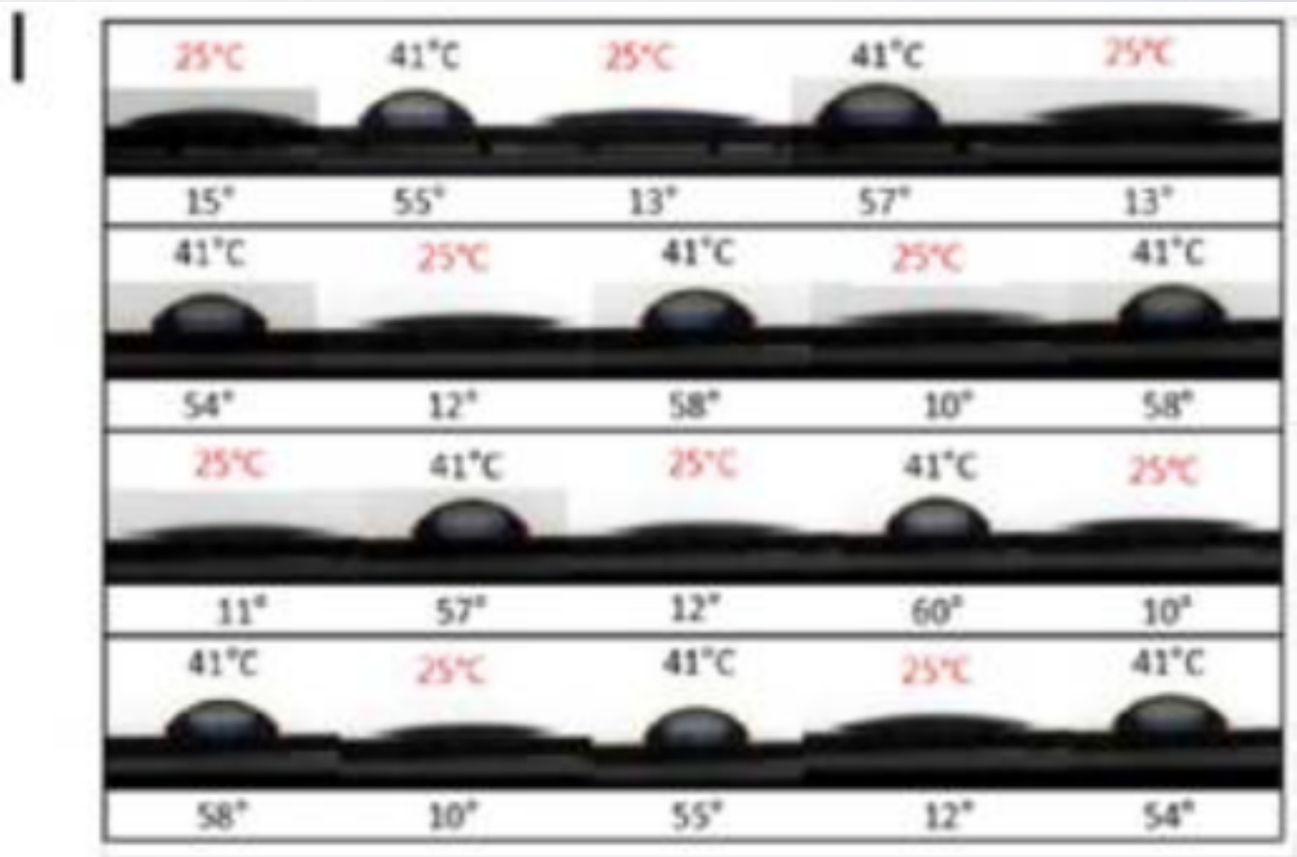


NTHP before and after PNiPAAm grafting

FT-IR spectra

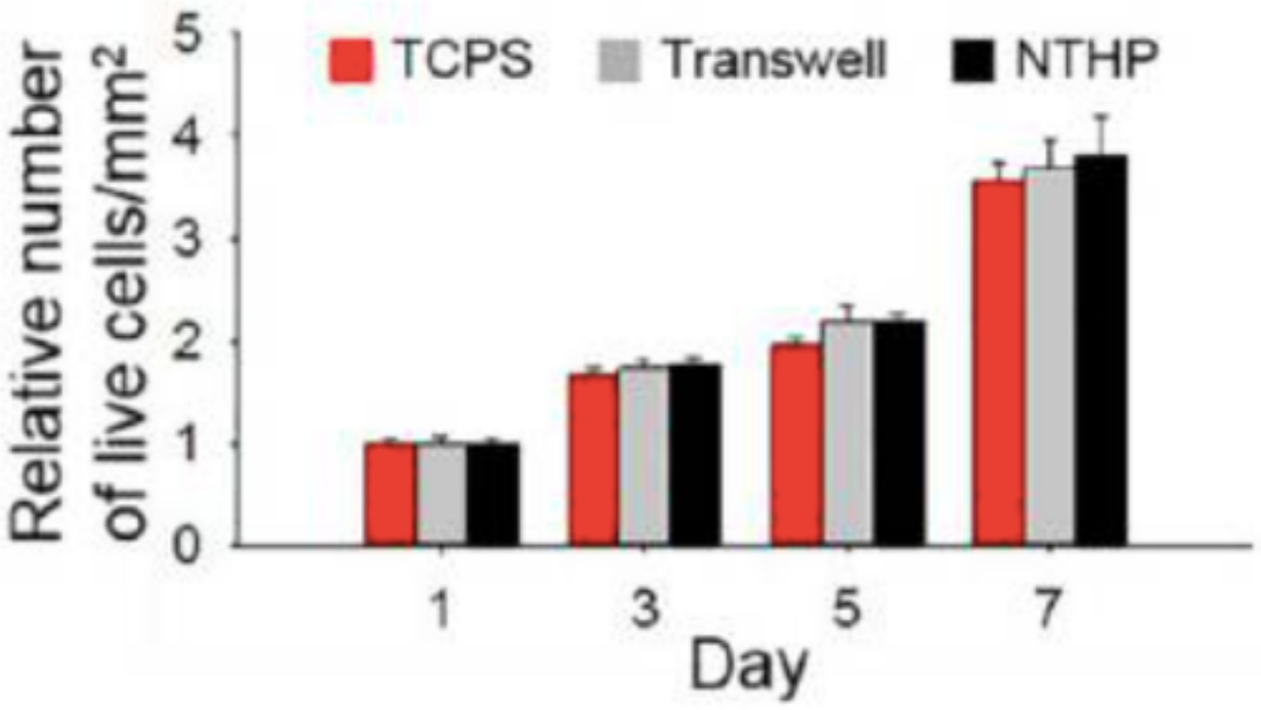
functional assay:
temperature dependence of hydrophobicity



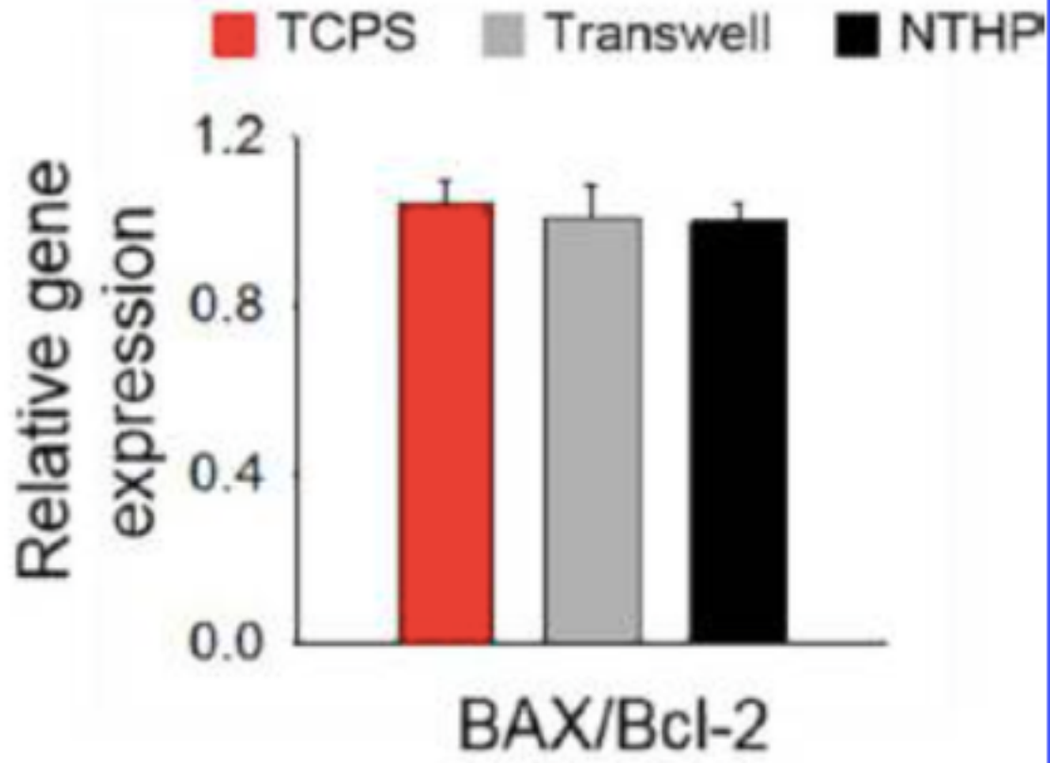


assessment of MSC proliferation and metabolic activity

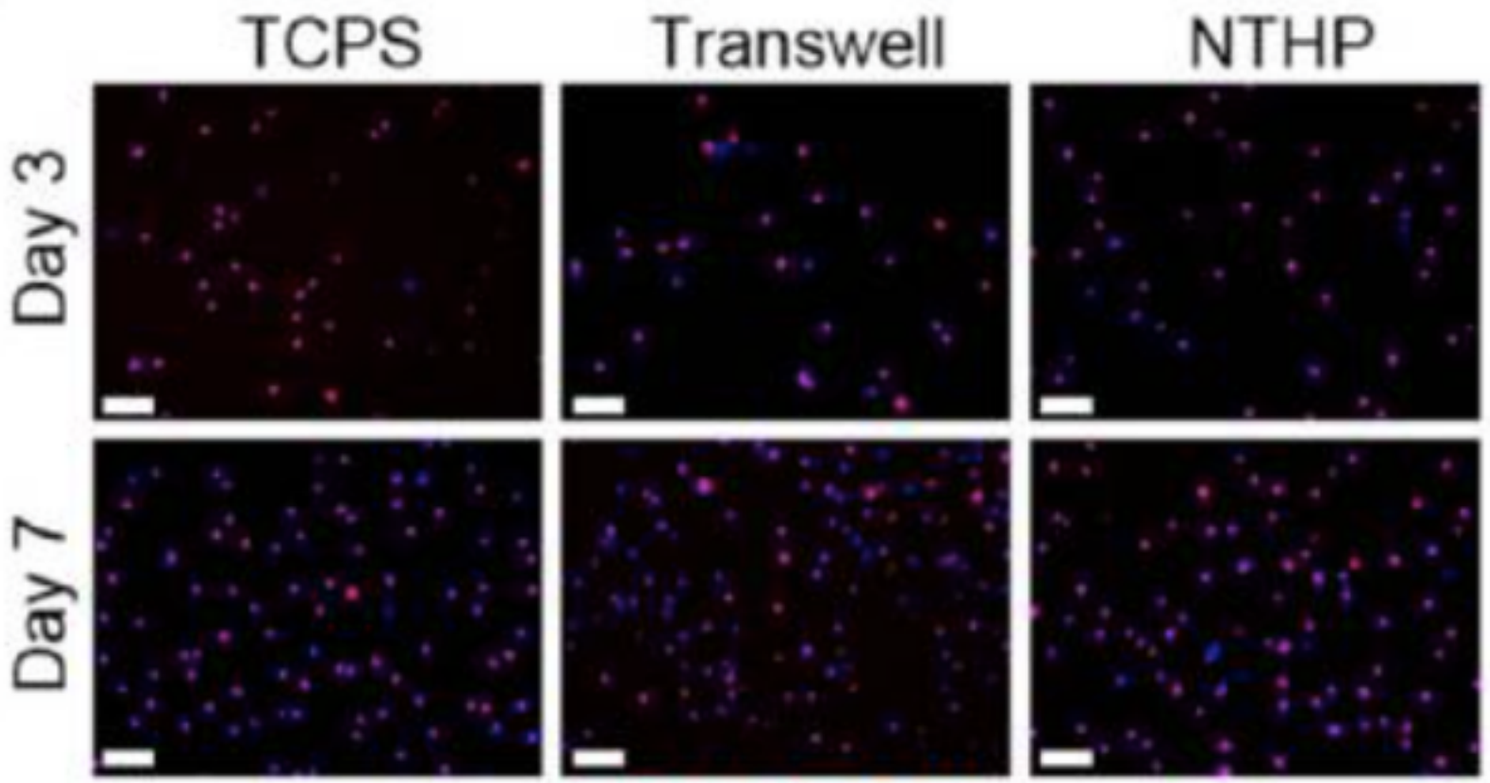
A

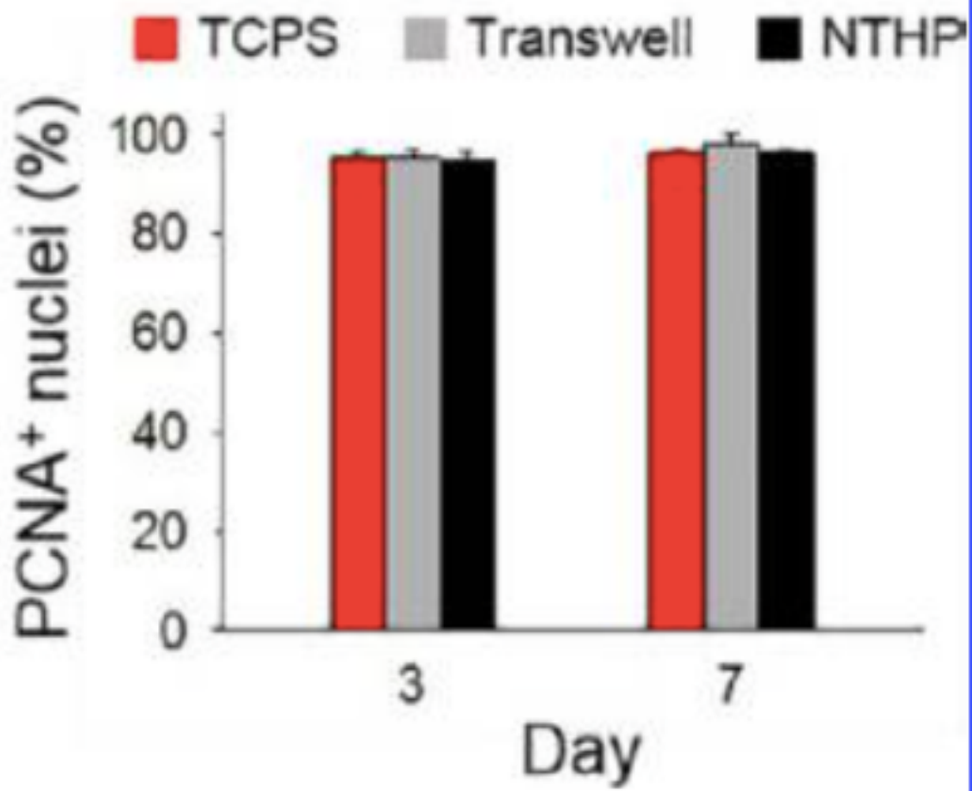


B

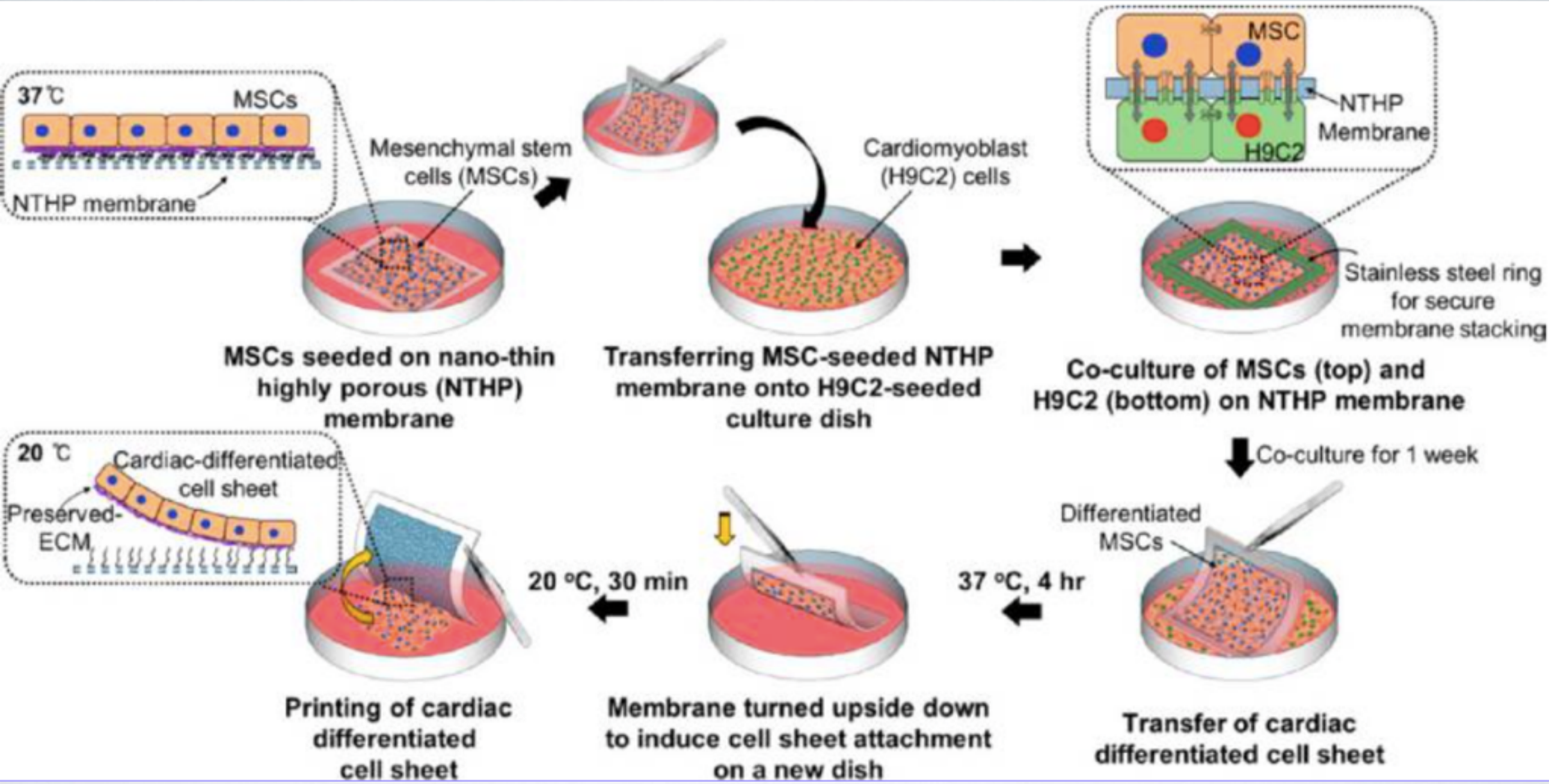


C

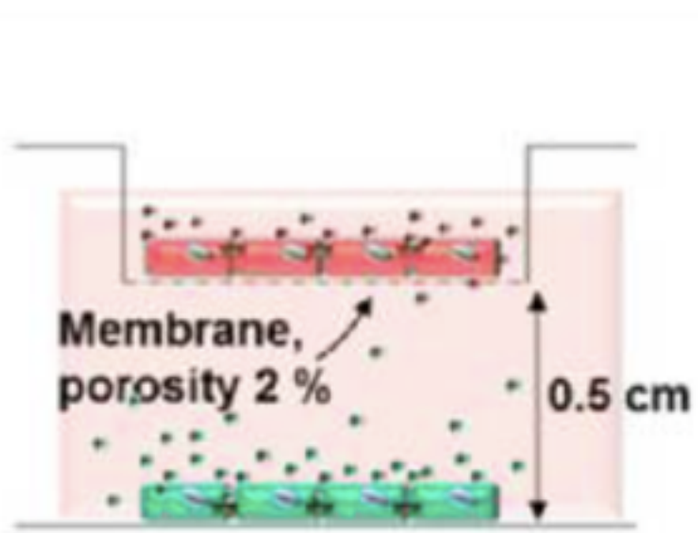
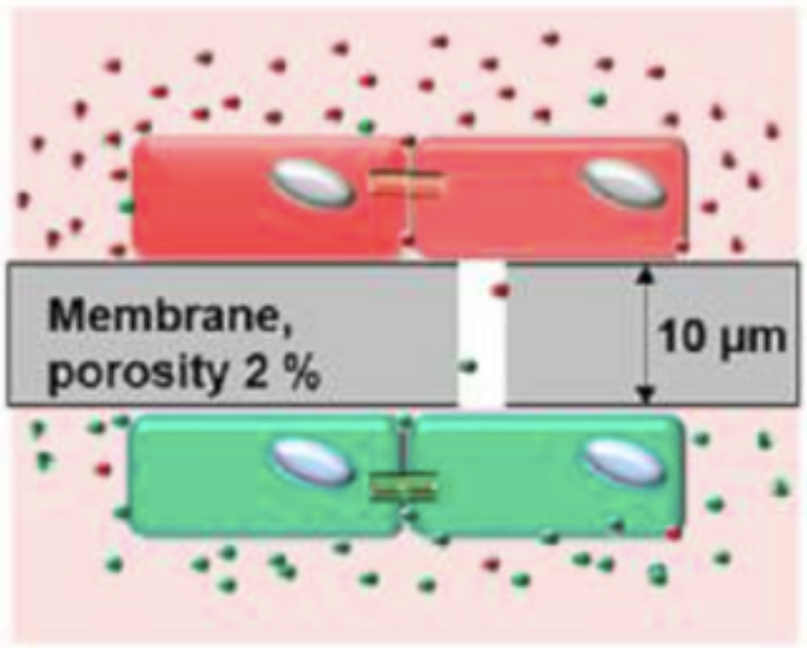
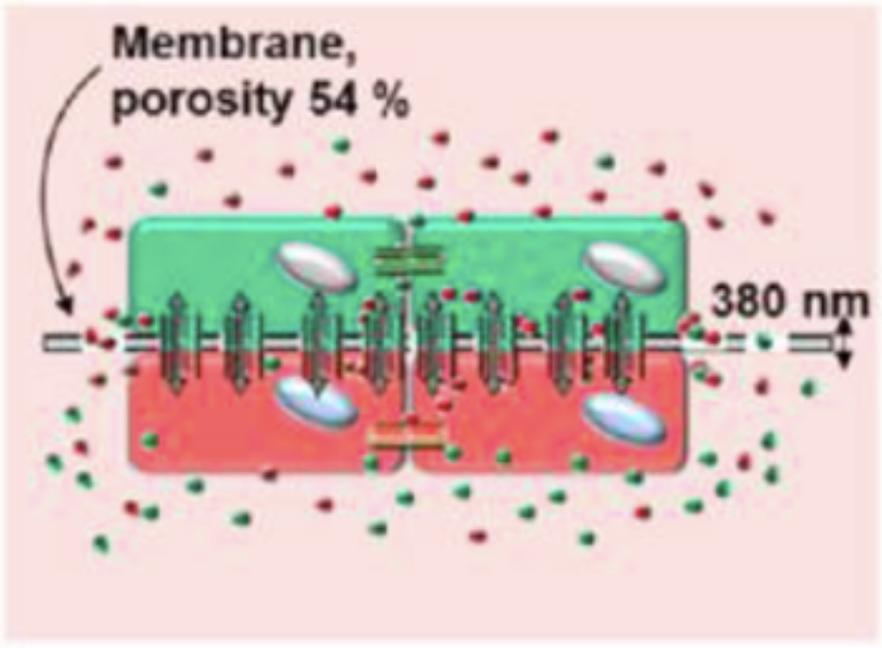




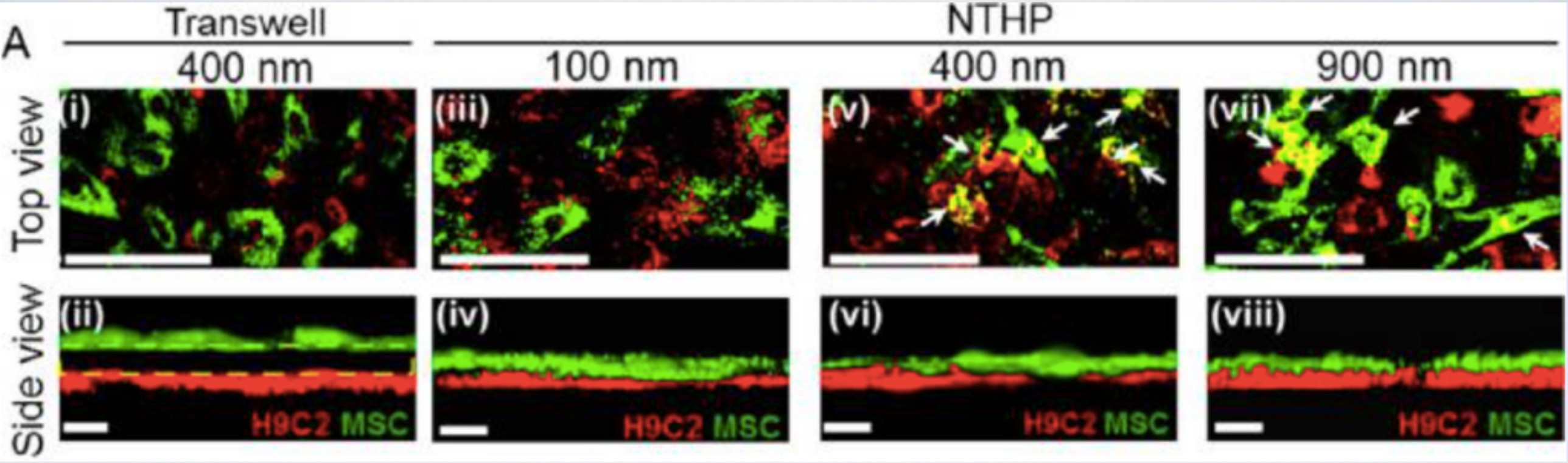
workflow of MSC/H9C2 co-culture



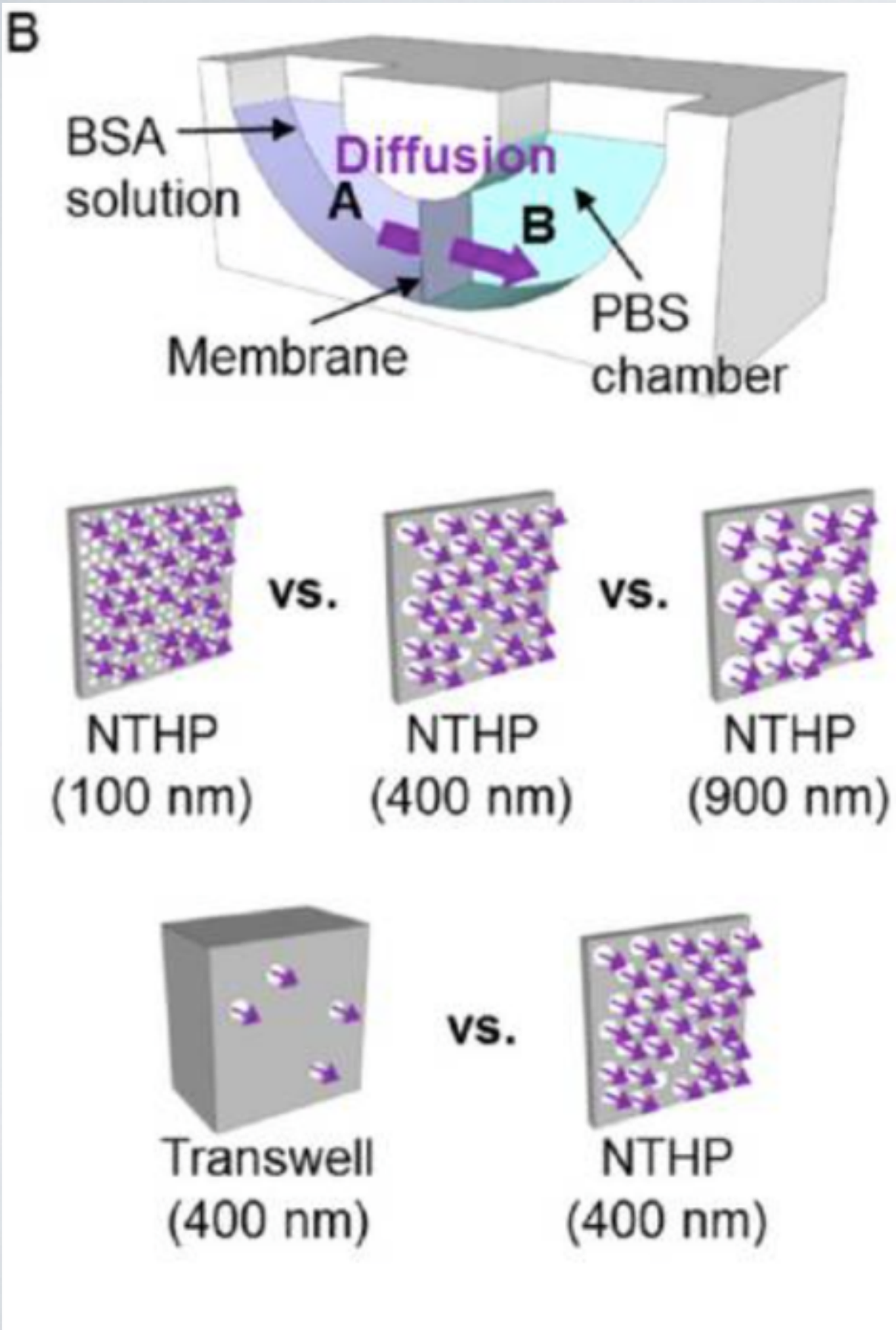
Transwell vs NTHP (in MSC/H9C2 co-culture)

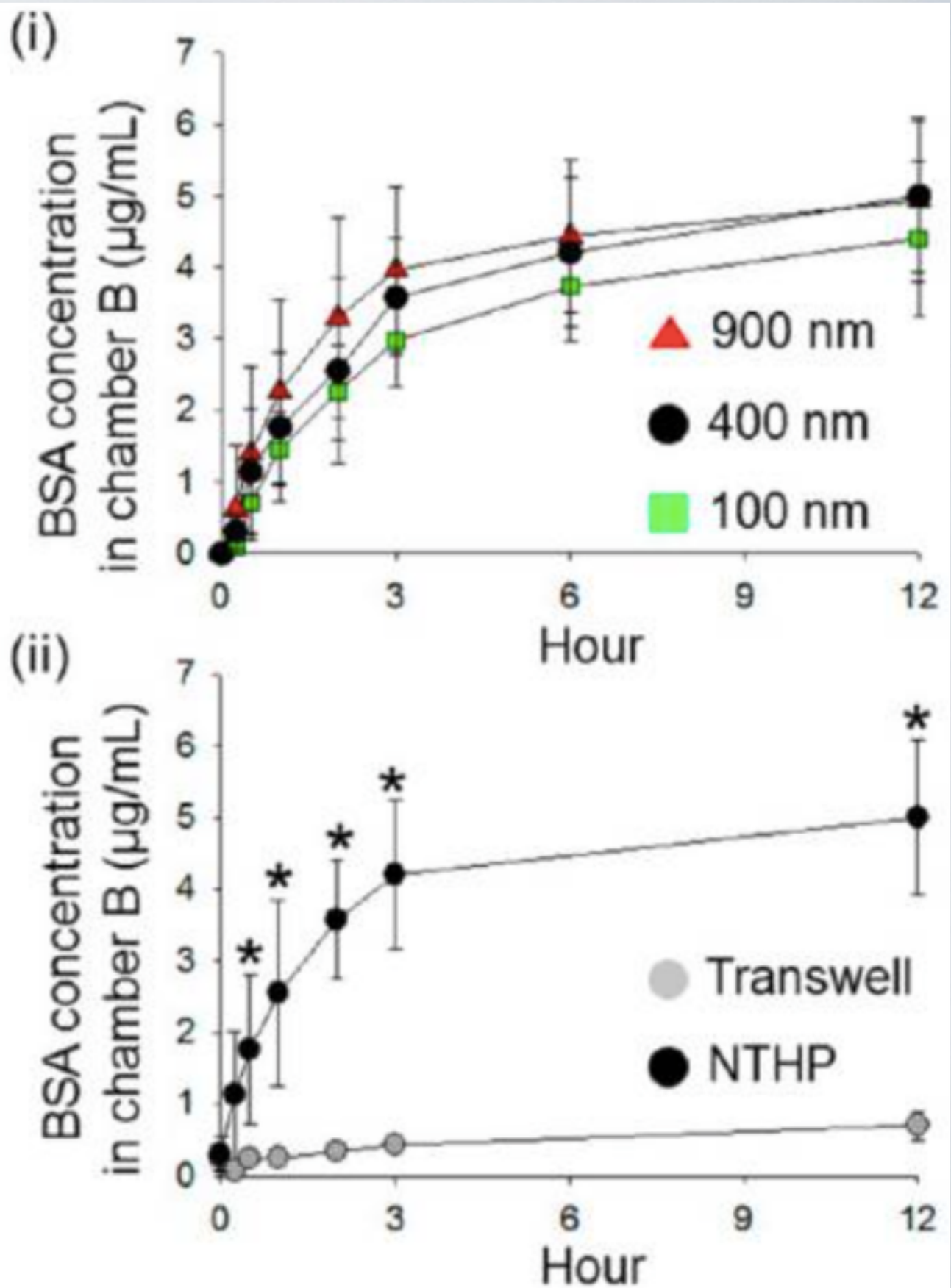
Indirect Co-Culture Using Transwell	Direct Co-Culture Using Transwell	Co-Culture Using NTHP Membrane
		
▪ Thick, less porous membrane ▪ No cell-cell contact ▪ Limited diffusion of cytokines		▪ Nano thin, highly porous membrane ▪ Effective cell-cell contact ▪ Enhanced diffusion of cytokines

MSC/H9C2 contact in co-culture

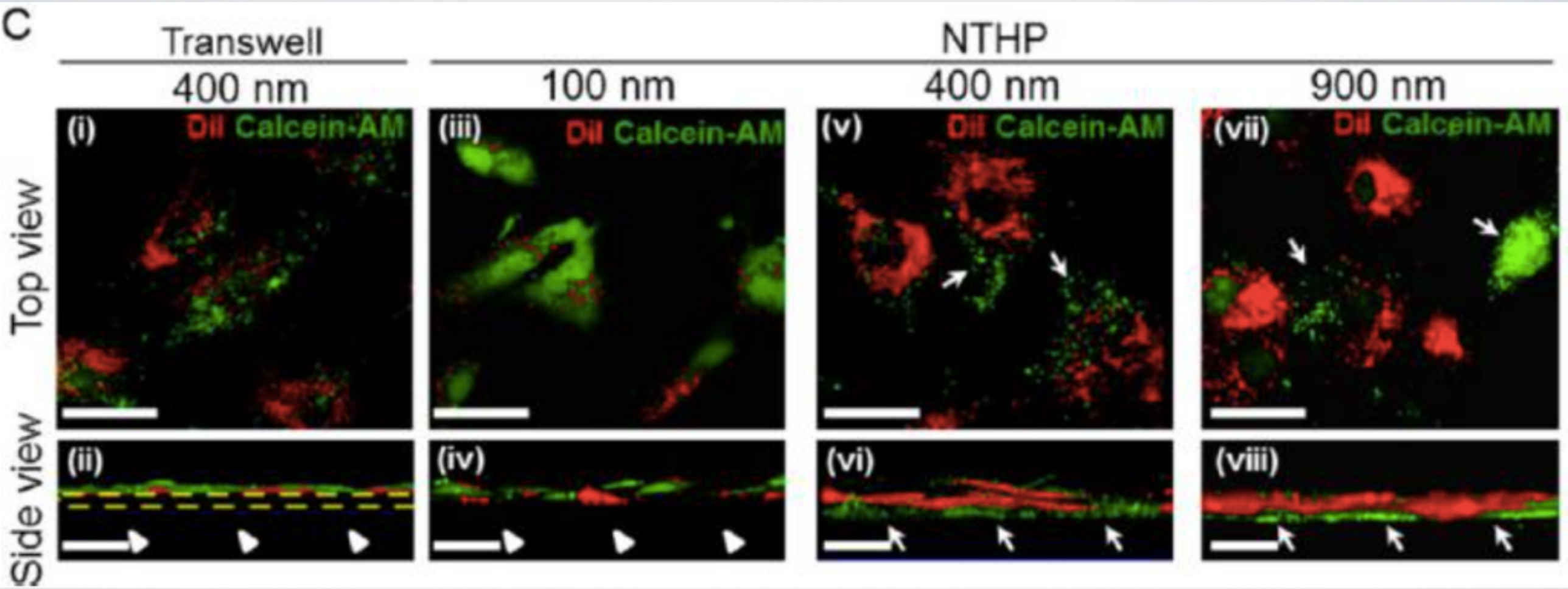


BSA diffusion across membrane





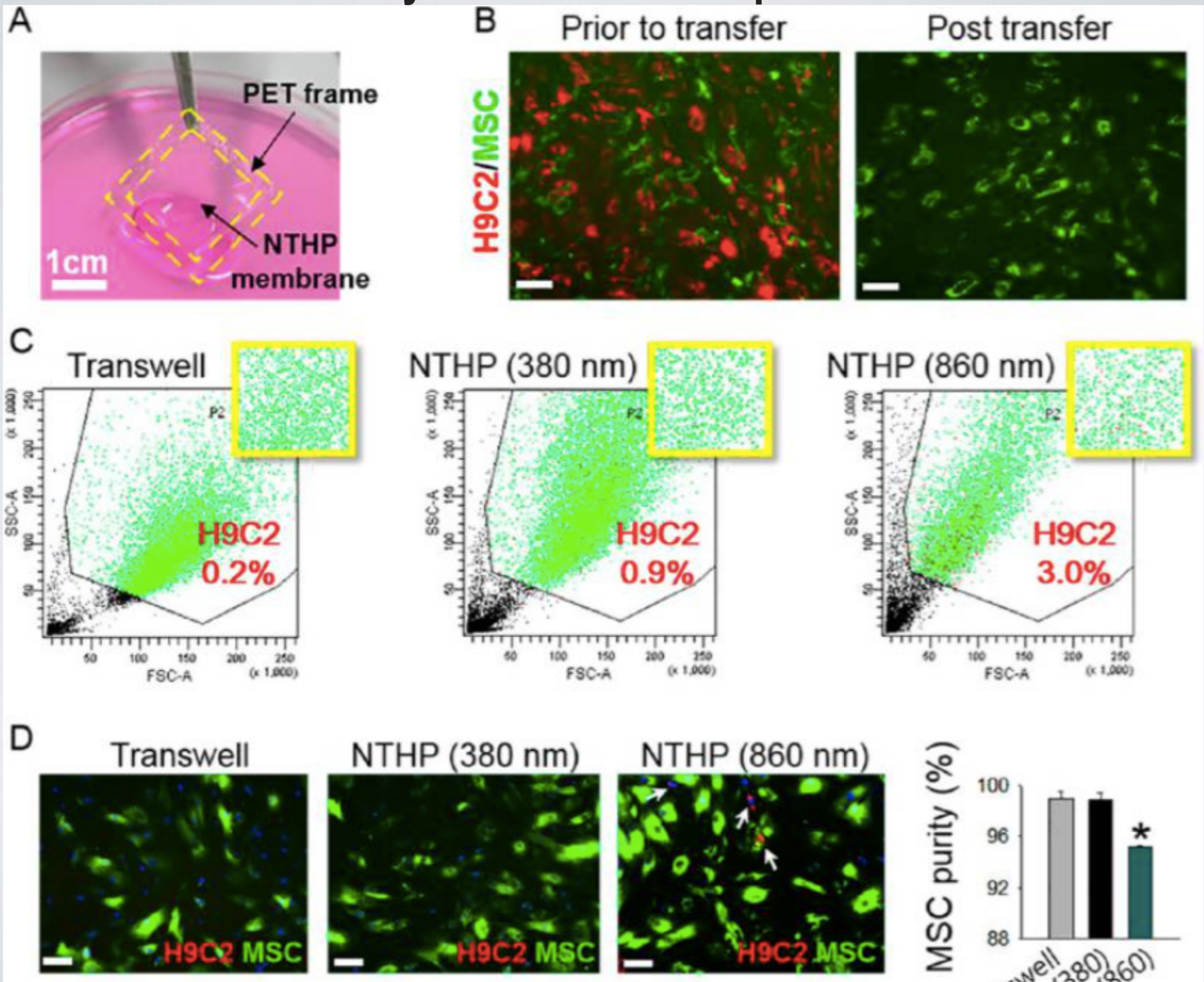
gap junctional transport



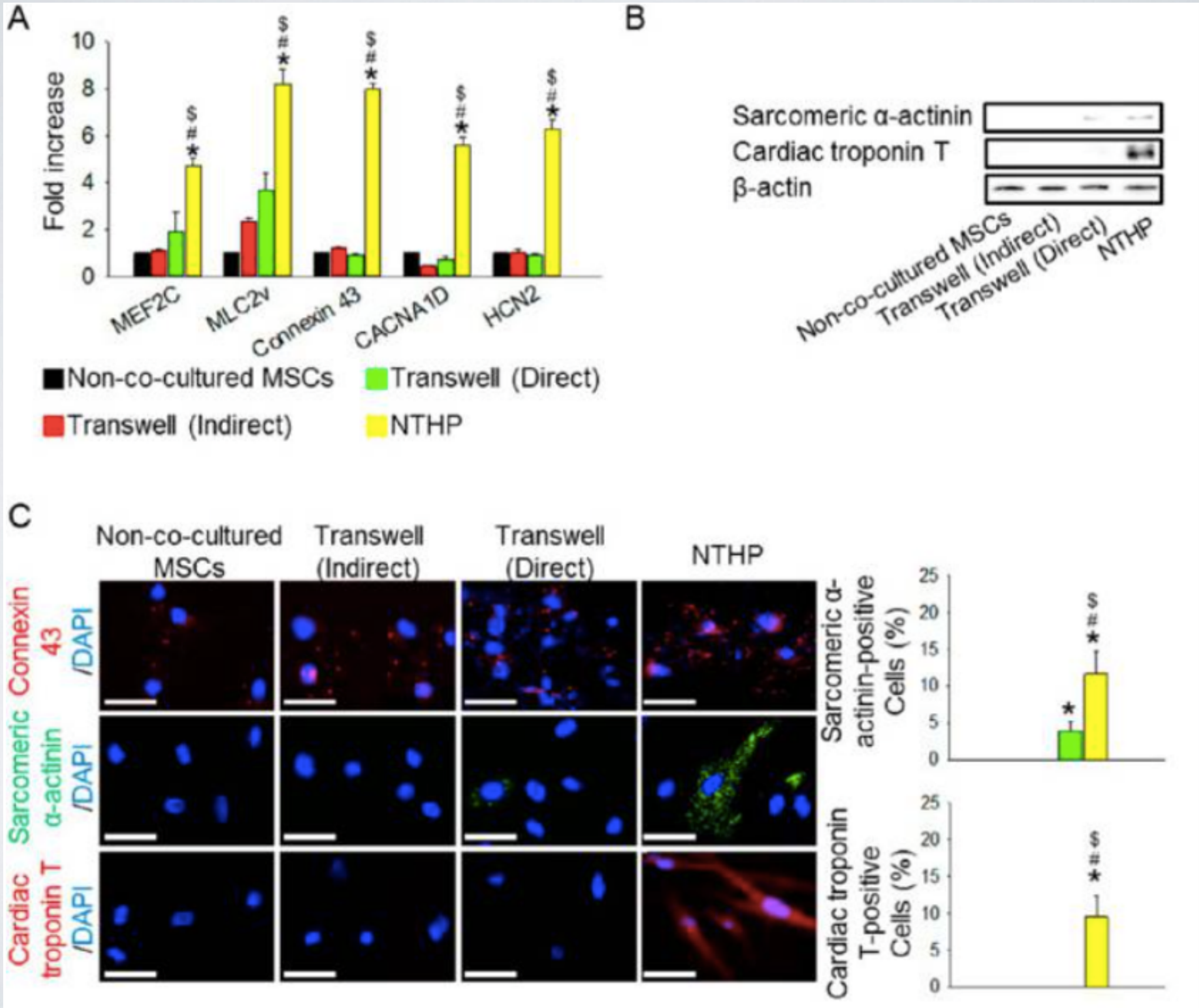
DiI: cell membrane marker, cannot transfer through cell-cell contact

Calcein-AM: becomes fluorescent after entering cell, and can only exit cell through cell-cell gap junction

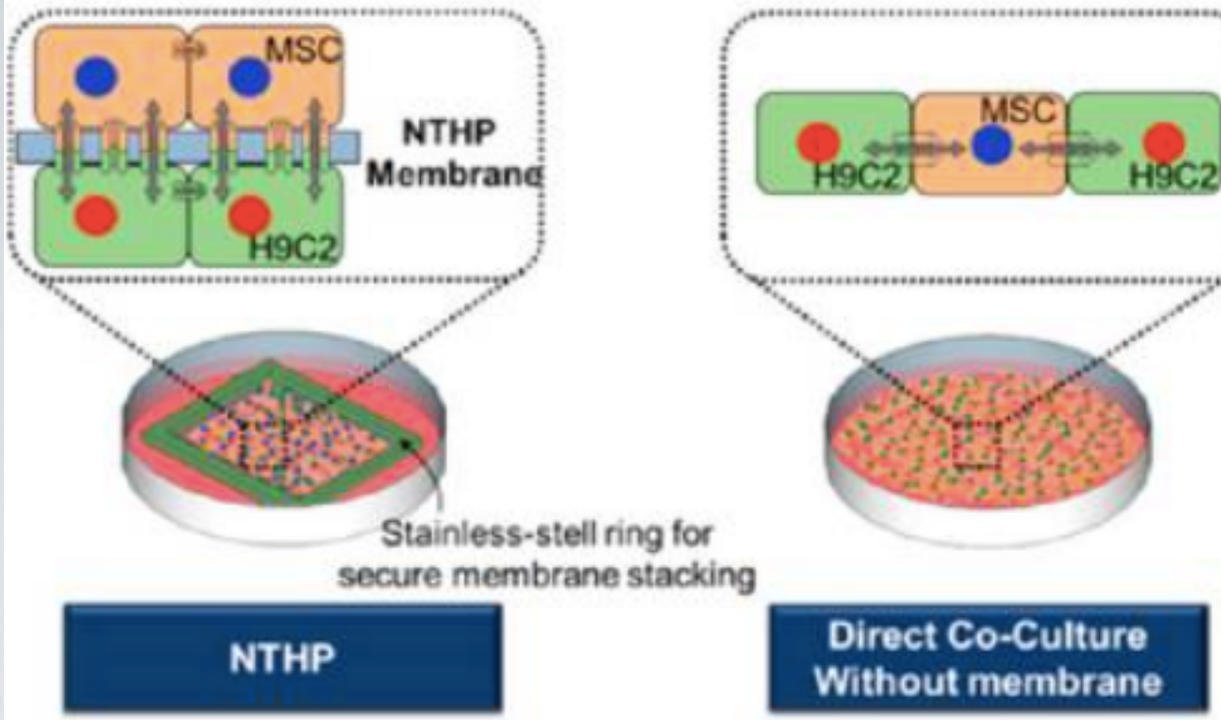
fidelity of MSC/H9C2 separation



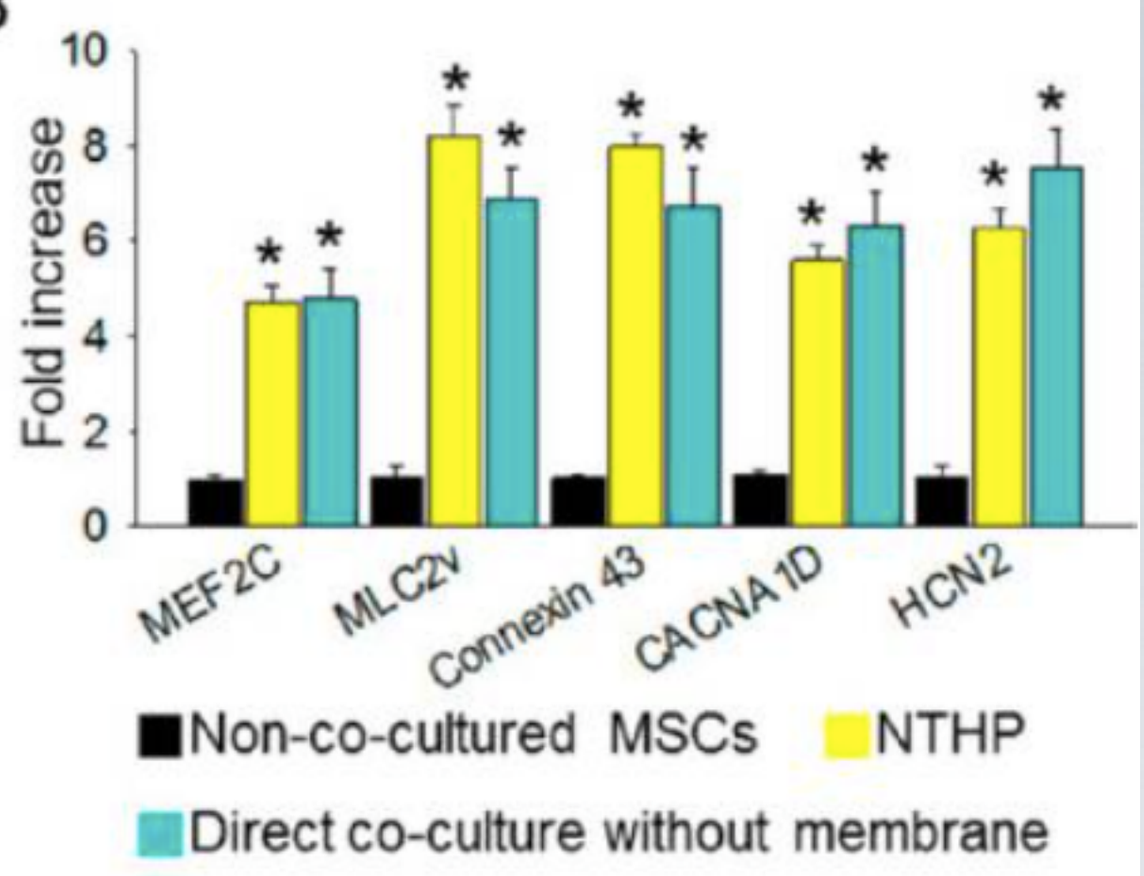
NTHP promoted higher degree of MSC differentiation in co-culture



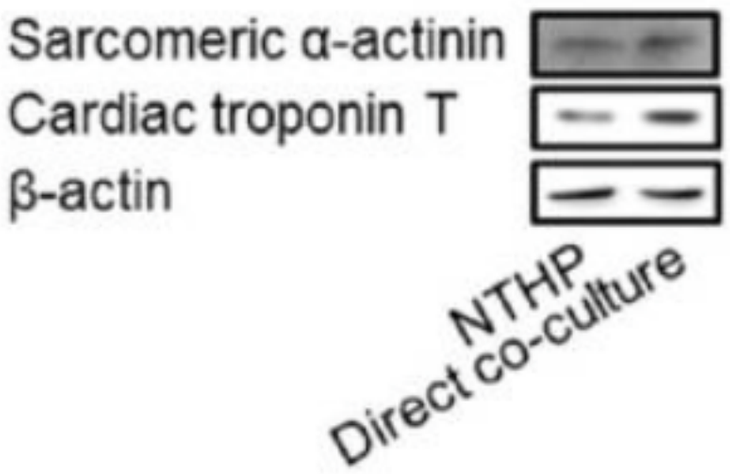
A


MSC
NTHP Membrane
H9C2
Stainless-stell ring for secure membrane stacking
NTHP
MSC
H9C2
H9C2
Direct Co-Culture Without membrane

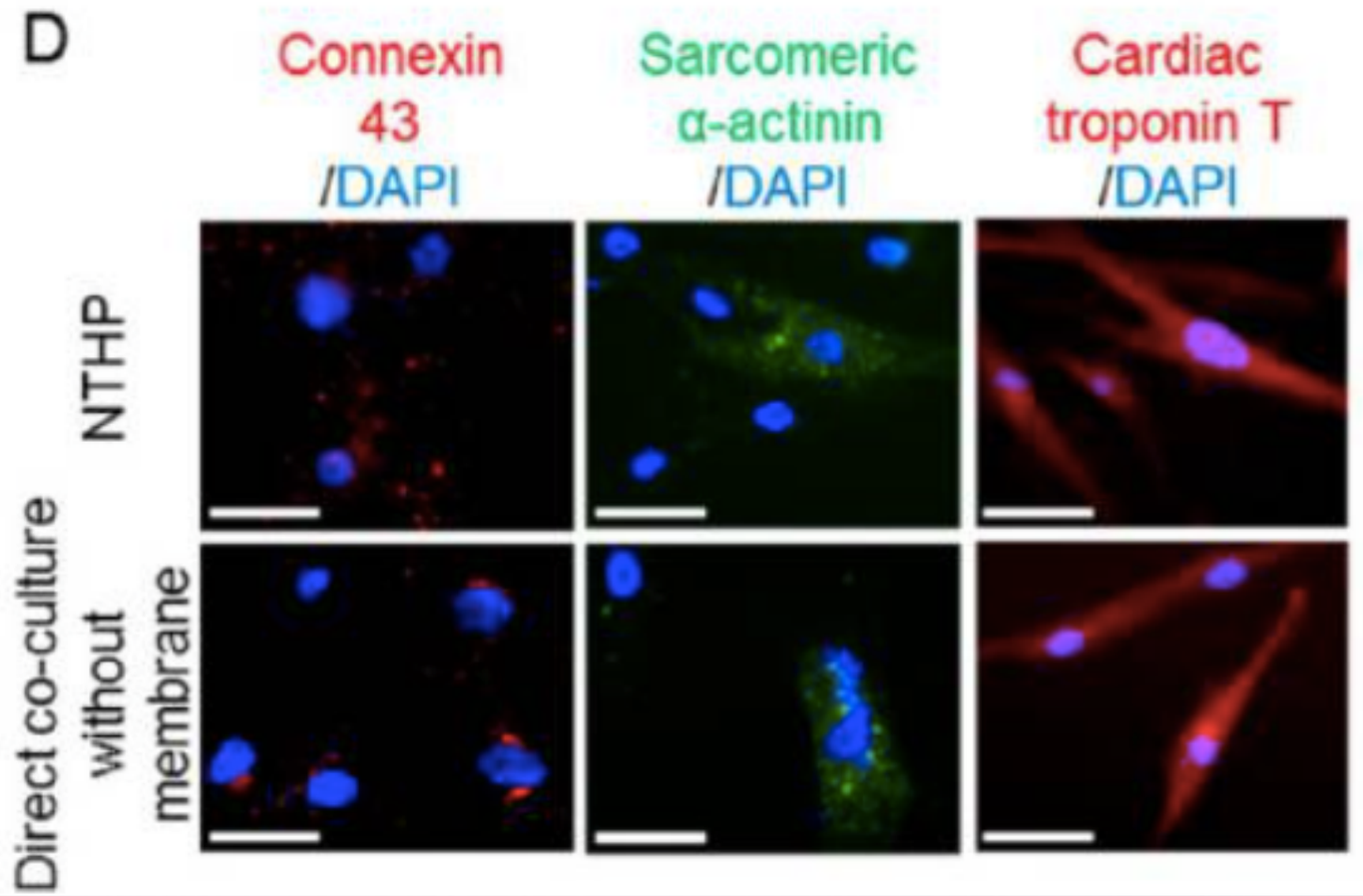
B


Fold increase
10
8
6
4
2
0
MEF2C
MLC2v
Connexin 43
CACNA1D
HCN2
Non-co-cultured MSCs
NTHP
Direct co-culture without membrane

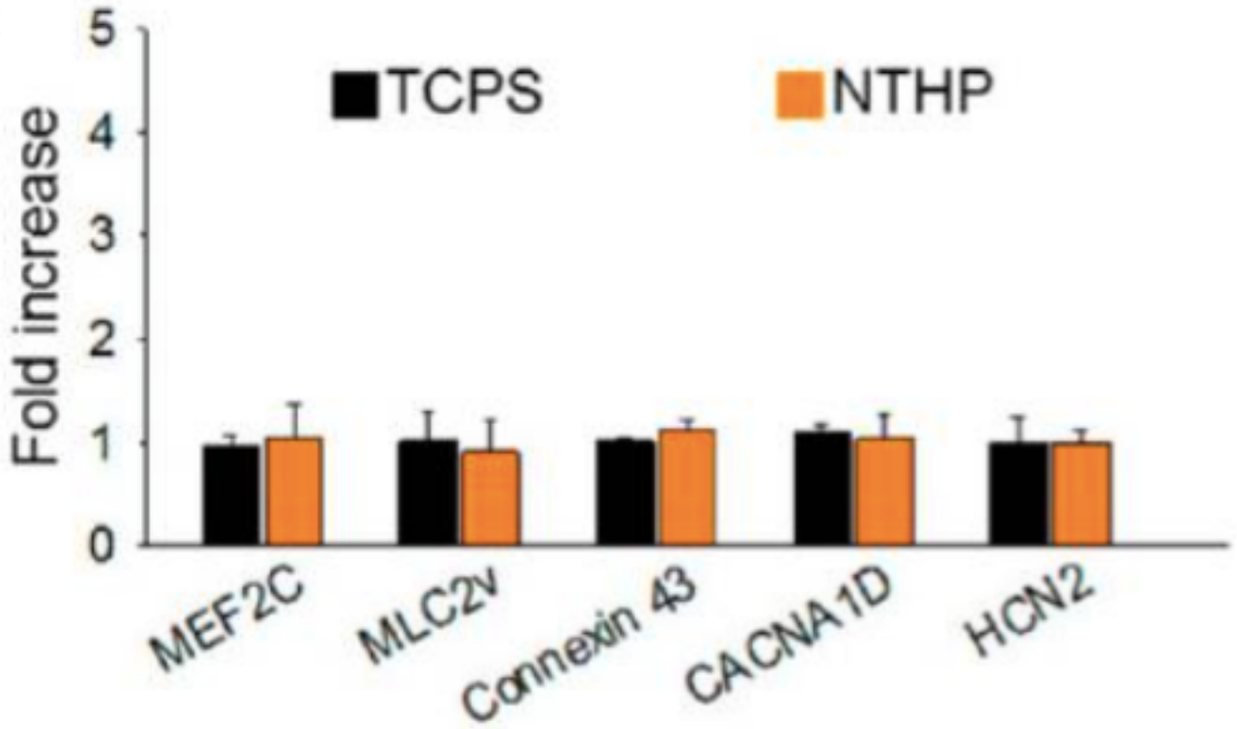
C


Sarcomeric α-actinin
Cardiac troponin T
β-actin
NTHP
Direct co-culture

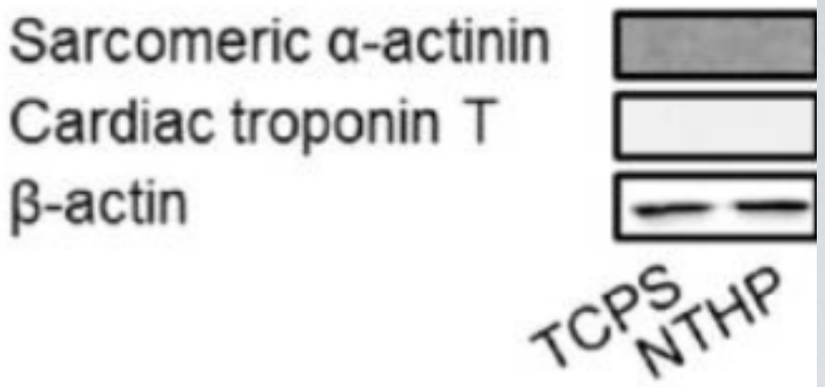
D


Connexin 43 /DAPI
Sarcomeric α-actinin /DAPI
Cardiac troponin T /DAPI
NTHP
Direct co-culture without membrane

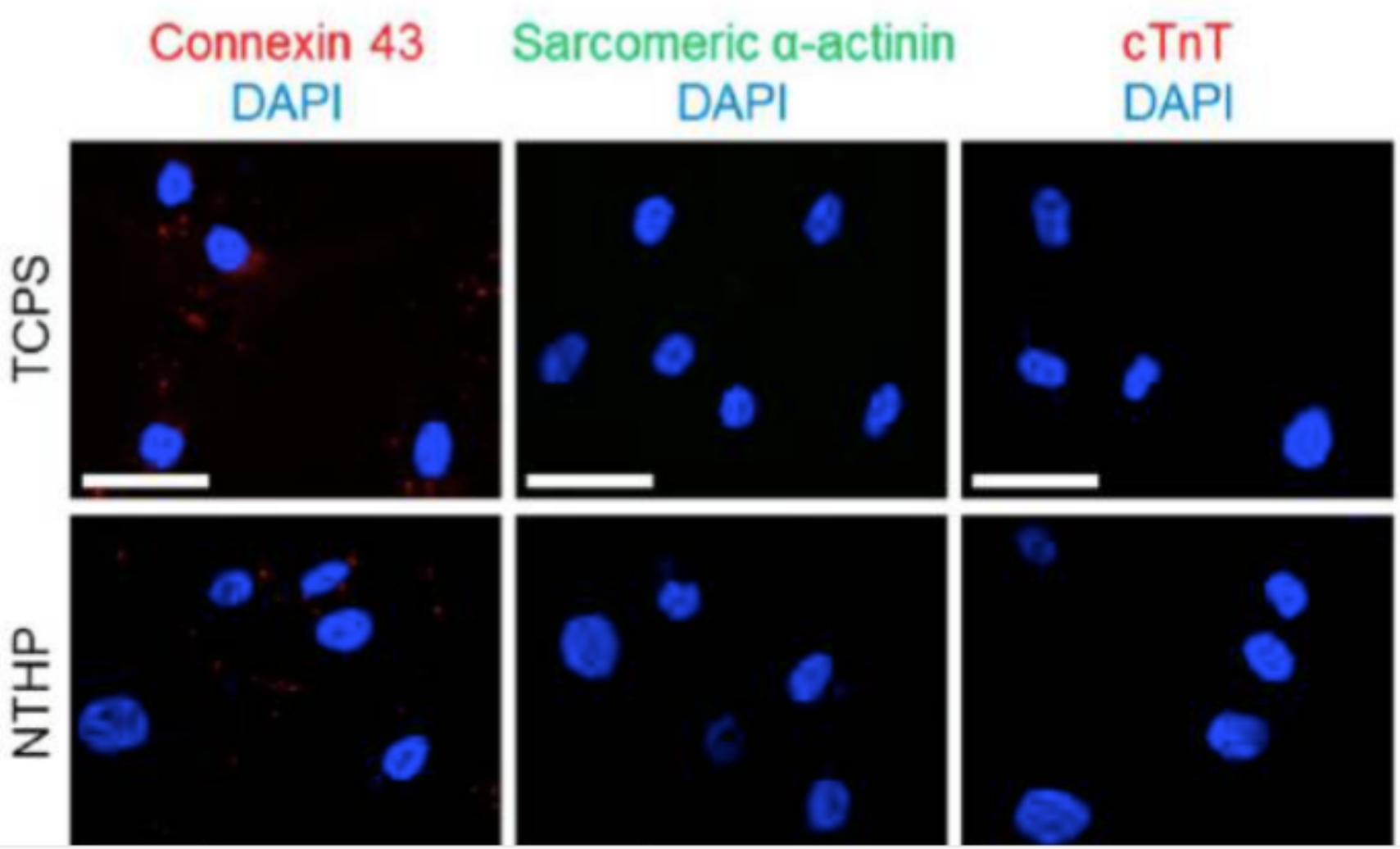
A


TCPS
NTHP
Fold increase
0
1
2
3
4
5
MEF2C
MLC2v
Connexin 43
CACNA1D
HCN2

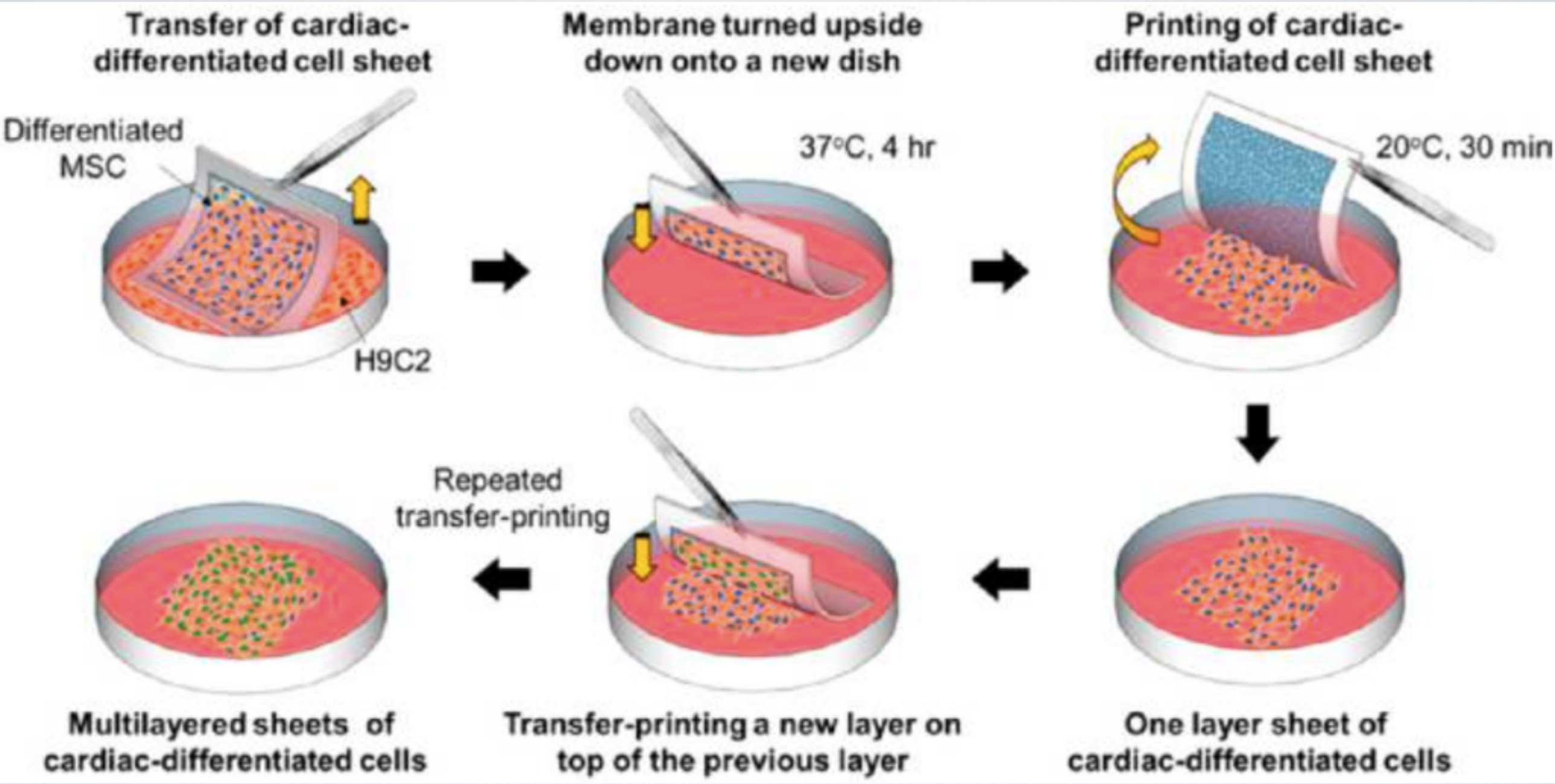
B


Sarcomeric α-actinin
Cardiac troponin T
β-actin
TCPS
NTHP

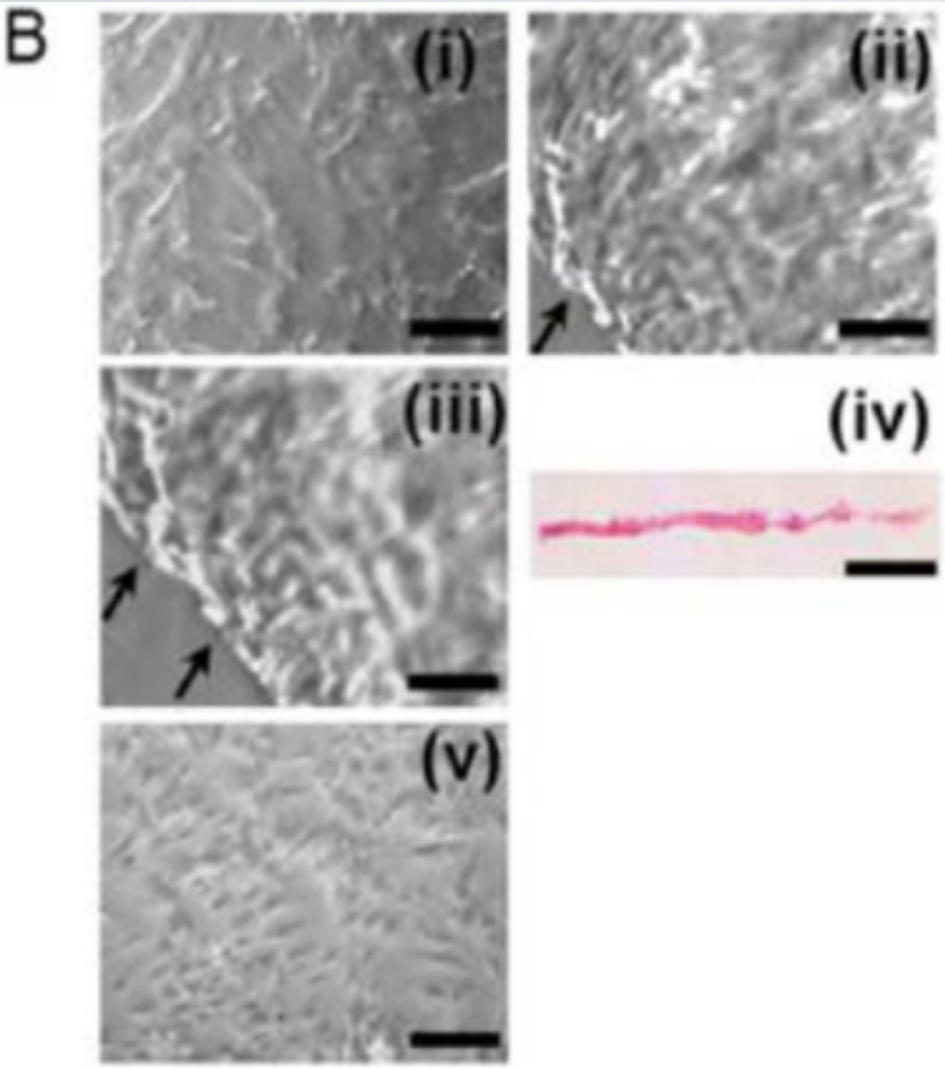
C

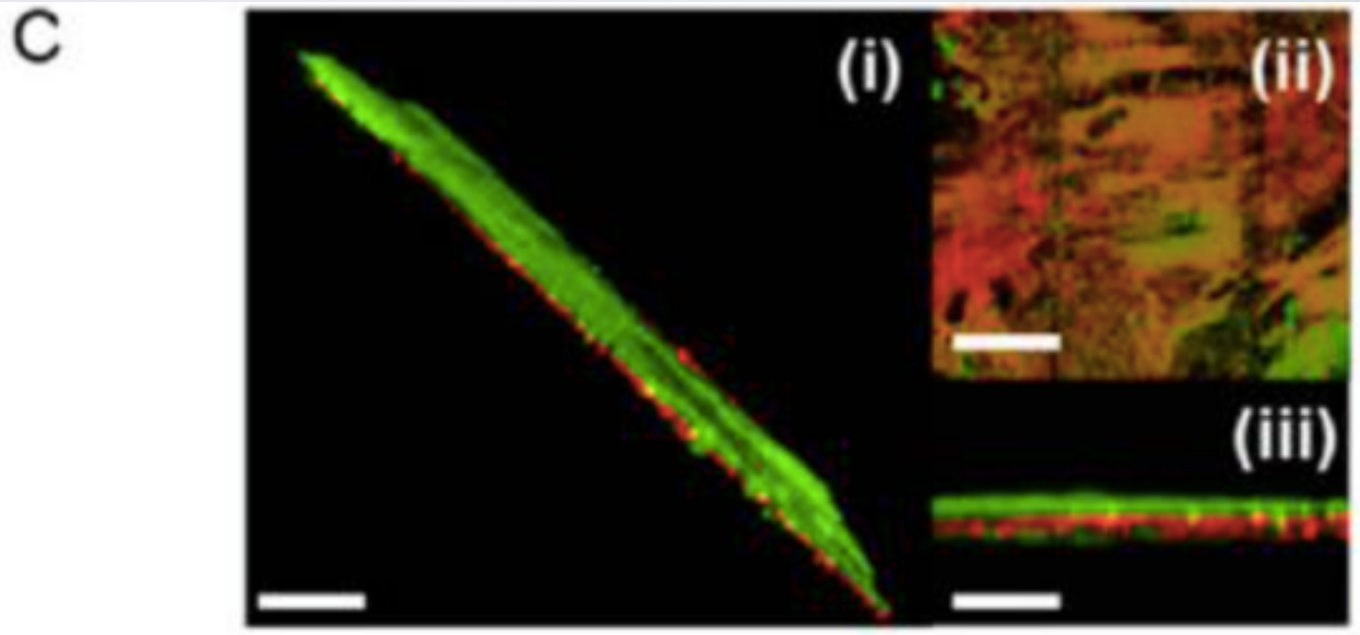

Connexin 43
DAPI
Sarcomeric α-actinin
DAPI
cTnT
DAPI
TCPS
NTHP

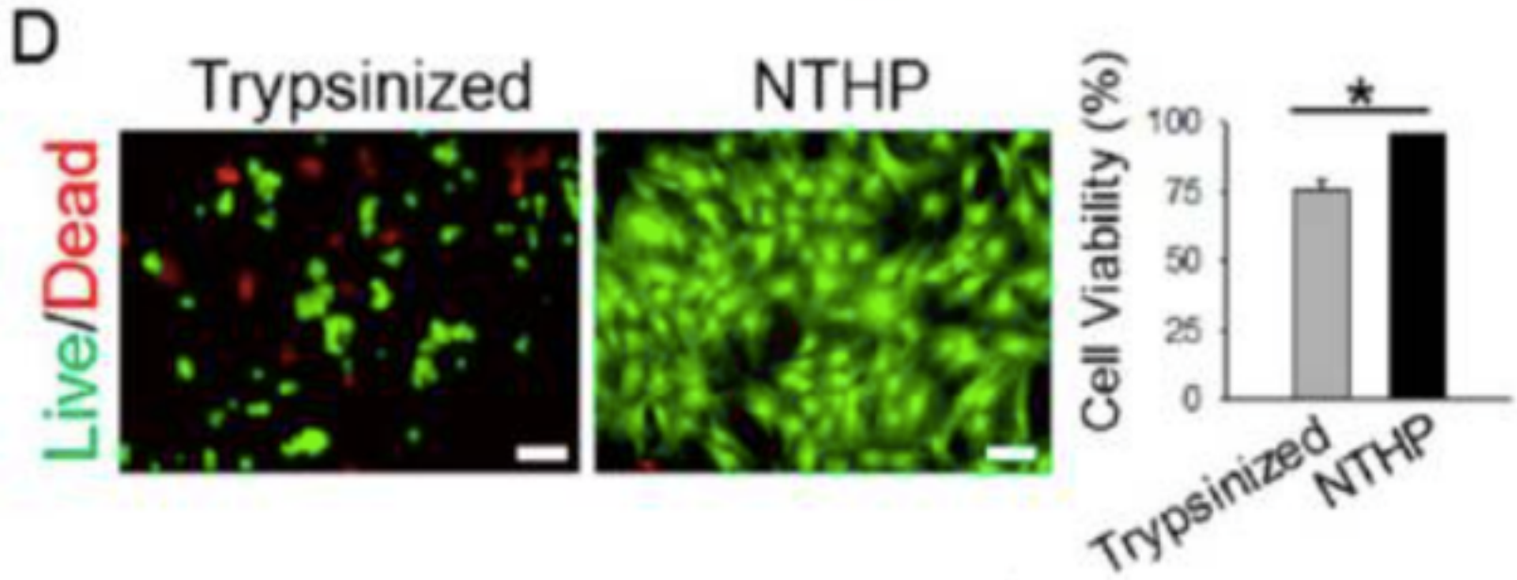
schematics of a multilayered culture



demonstration of proper cell detachment and multilayering







demonstration of ECM preservation post cell sheet detachment