Engineered Membrane Systems for Advanced Organotypic Tissues

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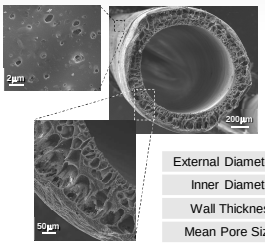
Aim

The design of a biomaterial that promote cell colonization and tissue regeneration represents a critical aspect and the major challenge in tissue engineering and regenerative medicine. Polymeric micro- and nano-structured membranes provide physical support, mechanical stimuli and biochemical cues able to modulate and affect the cell fate. Moreover, semipermeable microporous membranes allow the compartmentalization and the physical separation of cells, ensuring in the meantime their communication by the selective mass transfer of the secreted paracrine factors. In this work, two innovative culture strategies, by using engineered membrane systems for cell compartmentalization and colonization will be presented.

Vascularized 3D Liver Microtissue in a Compartmentalized PCL HF Membrane System

Poly(Ɛ-caprolactone) Hollow Fiber (PCL HF) membranes were synthesized and employed for the formation of a liver tissue on their extracapillary surface, and for the compartmentalization of endothelial cells in their lumen.

Membrane Properties



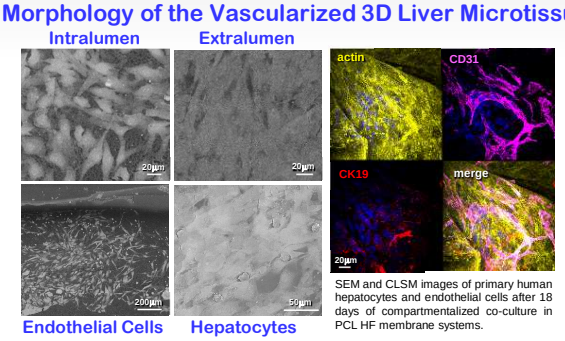
External Diameter	1537±85µm
Inner Diameter	1040±92µm
Wall Thickness	245±97µm
Mean Pore Size	0.39±0.007µm
Hydraulic Permeance	0.2396 [L/m2 h mbar]

Membrane Preparation

Membranes were prepared according to the dry-wet spinning method from solutions of PCL 15wt%, NMP 75wt%, Glycerol 10wt%.

✓ PCL HF membranes allowed the compartmentalization of cells in a microenvironment controlled at molecular level, ensuring the selective mass transfer of nutrients and molecules secreted by cells between both the two compartments.

Morphology of the Vascularized 3D Liver Microtissue

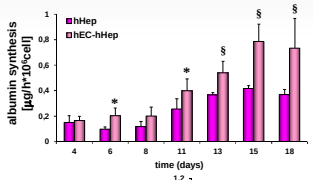


SEM and CLSM images of primary human hepatocytes and endothelial cells after 18 days of compartmentalized co-culture in PCL HF membrane systems.

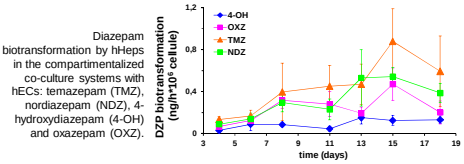
✓ PCL HF membranes provided a wide surface area for the adhesion and growth of cells in a small volume and in a 3D architecture, favoring cell-cell interactions, hepatocyte polarity and endothelial capillary-like structure formation.

★ The maintenance of a stable hepatic human phenotype for prolonged period is strictly required for therapeutic purpose and for drug testing in preclinical pharmacological research.

Liver Specific Metabolic Functions



Albumin synthesis by hHeps in homotypic and heterotypic co-culture with hECs in the compartmentalized PCL HF membrane systems. (*) p<0.01, (§) p<0.05, Student t'test.



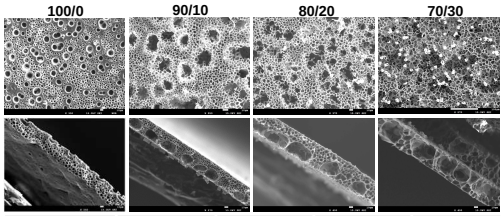
Diazepam biotransformation by hHeps in the compartmentalized co-culture systems with hECs: temazepam (TMZ), nordiazepam (NDZ), 4-hydroxydiazepam (4-OH) and oxazepam (OXZ).

✓ Human hepatocytes displayed relevant albumin synthesis as well as drug metabolism. In the compartmentalized co-culture systems, the liver specific functions were prominent in comparison to homotypic systems, thanks to the biochemical communications with human endothelial cells.

3D Membrane Scaffolds with Double Porosity as Niches for Human Mesenchymal Stem Cells (hMSC)

Poly (Ɛ-caprolactone) (PCL)/chitosan (CHT) blend membranes with double porous morphology were developed. The double porosity consists of surface macrovoids (big pores) which could be easily accessible for hMSCs invasion and proliferation, and interconnected microporous network to transfer essential nutrients, oxygen, growth factors between the macrovoids and throughout the scaffolds.

Membrane Properties



	100/0	90/10	80/20	70/30
Polymer wt (%)	15	14	14	10
Macrovoids diameter (µm)	13±4 (surface) 22±3 (bulk)	15±5 (surface) 34±10 (bulk)	21±11 (surface) 27±13 (bulk)	35±25 (surface) 46±4 (bulk)
Mean Flow Pore Diameter (µm)	0.022	0.069	0.049	1.55
Effective Surface Area (%)	88±1	84±1	74±2	69±2
Porosity (%)	39	46	48	55
Thickness (µm)	45±0.9	50±1	60±0.4	55±0.6
Tensile modulus E (N/mm²)	140.9±14.1 (dry) 131.6±2.2 (wet)	114.6±4.8 (dry) 58.5±4.1 (wet)	72.8±6.5 (dry) 29.5±0.8 (wet)	111.9±4.6 (dry) 10.1±2.7 (wet)
Ultimate Tensile Strenght (N/mm²)	8.3±0.7 (dry) 8.1±0.2 (wet)	3.7±0.4 (dry) 2.5±0.3 (wet)	2.4±0.2 (dry) 1.3±0.1(wet)	2.1±0.2 (dry) 0.5±0.2 (wet)
Elongation at break ε (%)	127.6±50 (dry) 146.9±68.4 (wet)	13.1±2.2 (dry) 17.6±8.8 (wet)	6.9±0.8 (dry) 15.8±5.1 (wet)	2.1±0.4 (dry) 9.1±1.2 (wet)

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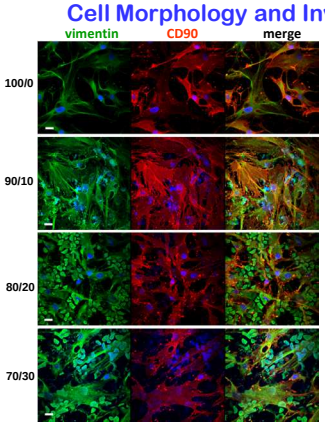
Membrane Preparation

Membranes were prepared using a liquid induced modified phase inversion technique from polymeric blend solutions of PCL and CHT at different ratio (100/0, 90/10, 80/20, 70/30). An isoporous membrane was used for inducing two different solvent-exchange which led to the double porous morphology.

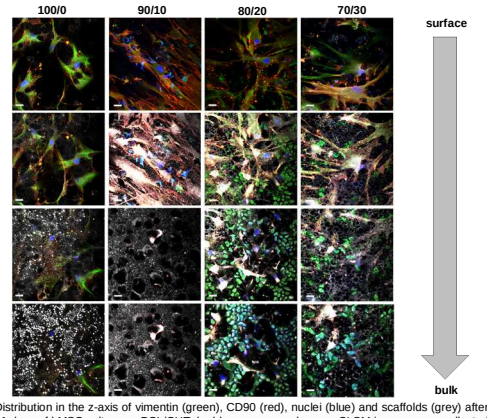
✓ By varying the PCL/CHT blend composition the mean macrovoid size, surface morphology and membrane properties changed. Membranes exhibited macrovoids connected with each other through a microporous network; macrovoids size increased by increasing the CHT wt%.

★ New possibility for stem cell based tissue engineering are open with these membranes.

Cell Morphology and Invasion in the Double Porous Membranes



CLSM images of hMSCs after 21 days of culture on PCL/CHT double porous membranes. Scale bar 20µm.



Distribution in the z-axis of vimentin (green), CD90 (red), nuclei (blue) and scaffolds (grey) after 21 days of hMSC culture on PCL/CHT double porous membranes. CLSM images were collected from the surface to the bulk in the z-scan mode at step size intervals of 5 µm. Scale bar 20 µm.

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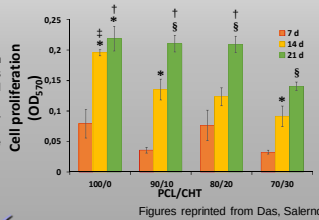
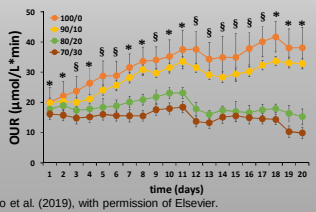
✓ Cells adhered on the surfaces of PCL/CHT 100/0 and PCL/CHT 90/10 membranes, that are characterized by a high effective surface area and small macrovoids. PCL/CHT 80/20 and PCL/CHT 70/30 membranes with large macrovoids and low effective surface area entrapped cells inside macrovoids.

hMSCs Proliferation and Oxygen Uptake

Cell proliferation of hMSCs on PCL/CHT double porous membranes. ANOVA followed by Bonferroni t'test ($p < 0.05$): (*) vs 7 days, (§) vs 7 and 14 days, on the same substrate; (†) vs 70/30, (‡) vs all, at the same day of culture.





Oxygen uptake rate of hMSCs on PCL/CHT double porous membranes. ANOVA followed by Bonferroni t'test ($p < 0.05$): (*) 100/0 vs all, 90/10 vs 80/20 and 70/30, and 80/20 vs 70/30; (§) 100/0 vs all, 90/10 vs 80/20 and 70/30, at the same day of culture.

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✓ The scaffolds created a permissive environment for hMSC adhesion and invasion and ensured an adequate diffusive mass transfer of nutrients and metabolites, which are essential for the long-term maintenance of cell viability and functions. A relationship between hMSCs proliferation and oxygen uptake rate with surface mean macrovoid size and effective surface area was found.

Conclusions

Both the engineered membrane systems can provide selective cell co-culture models for different tissues where cells can adhere on the surface and simultaneously can be compartmentalized or entrapped in macrovoids, ensuring the biochemical cross-talk, which is necessary for recapitulating physiological functions.

References

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✓ Das P, Salerno S, et al. *Colloids Surf B-Biointerfaces,* 2019; 184: 110493.