Effect of Porous and non-Porous PCL Fiber Meshes on $CaCO_3$ Crystallization Through Gas Diffusion Method

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1. Experimental Procedures

1.1. Materials and reagents

Chemicals used in this work were purchased from Sigma-Aldrich and Merck. Solvents were of the highest available grade. Calcium chloride, ethanol, hydrochloric acid, sodium hydroxide (analytical grade) and tris(hydroxymethyl) amino methane (TRIS) were obtained from Merck. Ammonium hydrogen carbonate (NH_4HCO_3) was from J.T. Baker and methylene chloride (CH_2Cl_2 > 99.5%) was from Sigma-Aldrich. Ultrapure water (18.2 MΩ) from water system LaboStar^{TM} 4-DI/-UV was used for all solutions involved in the $CaCO_3$ crystallization. Glassware cleaning was performed by washing with neutral detergent, rinsing with ultrapure water, sonicating in cold ethanol for 5 min, rinsing with ultrapure water, submerging three times in piranha solution, rinsing again with ultrapure water, washing with acetone and drying under vacuum in an oven at 20 °C. The piranha solution was prepared by mixing equal parts of H_2O, HNO_3 and H_2O_2 solutions (1:1:1, v/v/v).

1.2. Gas diffusion crystallization of $CaCO_3$

A set of *in vitro* gas diffusion (GD) crystallization of $CaCO_3$ essays were performed on pieces of PCL electrospun fiber meshes (2x3 mm) at 20 °C for 24 h. Indeed, GD crystallization was carried out with non and porous- random and aligned electrospun PCL fiber meshs as 3D template and compared with melted PCL and without template as negative and positive controls. Similar GD crystallization experiment has been reported in our previous works.[1,2] Briefly, a chamber consisting of an 85 mm Petri dish having a central hole (8 mm) in its bottom was glued to a cylindrical vessel 50 mm in diameter and 30 mm high. Inside the chamber, polystyrene micro-bridges were filled with 35 ml of 200 mM $CaCl_2$ solution in 200 mM TRIS buffer. The cylindrical vessel contained 3 ml of 25 mM NH_4HCO_3 solution. The $CaCO_3$ crystals results from the diffusion of CO_2 into the buffered $CaCl_2$ solution. In the case of mineralization assays with electropsun PCL fibers, the crystallization of $CaCO_3$ occurs on the surface of the PCL meshes.

Finally, the $CaCO_3$ crystals were washed with deionized water, dehydrated with growing concentration of ethanol from 50% to100% and dried at room temperature. All the resulting crystals floating in the $CaCl_2$ solution was removed in order to avoid their deposition on the PCL fiber meshes. The saturation of CO_2 gas in the sealed crystallization chamber is constant throughout the *in vitro* experiment due to the fact that the amount of volume and concentration of NH_4HCO_3 allows to achieve the thermodynamic equilibrium allowing the GD mineralization method to be reproducible. Once the experiment is finished, still NH_4HCO_3 solution remains in the cylindrical vessel. The crystal density predominance was determined based on the number of crystal population of vaterite and calcite crystals accounted by using lower magnification field at 70x. All crystallization tests were performed in duplicate (Figure S1).

1.3. Preparation of PCL fiber meshes by electrospinning

Aligned and random PCL fibers meshs with and without porous were obtained from 18% (w/v) PCL solution prepared using ethyl acetate/acetone 3:1 (v:v) mixture with flat (30×30 cm) or rotating (10 cm in diameter) collectors, respectively. Porous PCL meshes from 18% PCL solution in ethyl acetate/DMSO 1:9 (v:v) mixture were prepared. For all PCL fiber meshes, PCL (Mw, 80,000, Sigma-Aldrich) in an eStretching LE-10 Fluidnatek® equipment was utilized. The control of PCL surface topology was achieved by using the following parameters: 16 kV, solution flow rate of 1200 µl/h, 15 min, nozzle-collector distance from between 15 and 18 cm, and rotating speed of 2000 rpm in a warm (33°C) room (Figure S2).

1.4. Contact angle measurements of PCL fiber meshes

Water and crystallization solution contact angle [°] measurements were performed by using a DataPhysics optical contact system OCA 15EC (DataPhysics instruments GmbH, Filderstad, Germany) on melted PCL, glass, non- and porous- random and aligned electrospun PCL fiber meshes surfaces with a drop volume of 11 µL (Figure S3). In order to complete the physicochemical characterization and to determine

the hydrophobic/hydrophilic character of all electrospun polymer meshes, a drop liquid sample of ultra-pure water and the $CaCl_2$ in a TRIS buffer pH 9.00 solution on PCL fiber surface was deposited.

1.5. FTIR analysis of PCL fiber meshes and PCL samples

FTIR analyses were performed by using an Interspectrum Interspec p/n 200-X instrument to characterize spectroscopically the non- and porous- random and aligned electrospun PCL fiber meshes, melted PCL and the electrospun polymer solution.

1.6. Surface BET analysis

Specific surface area of the different PCL substrates used as template was measured by the N_2 method of Brunauer–Emmett–Teller (BET), and the pore size was calculated by means of the Barrett, Joyner and Halenda analysis of an N_2 adsorption/desorption isotherm at 273.0 K using outgas time of 1.0 h and analysis time between 119.8 min to 137.7 min in an automatic analyzer (Quantachrome Nova Station A) instrument. This equipment allows single or multipoint surface area analysis, adsorption and desorption isotherms, pore volume and pore size distribution. In addition, multi-point BET, BJH adsorption/desorption summaries of different PCL substrate are provided in the Table S1. Table S1 presents the specific surface area results obtained through the BET isotherm for each PCL mesh substrate.

2. Results and Discussion

2.1 Figure S1-S4

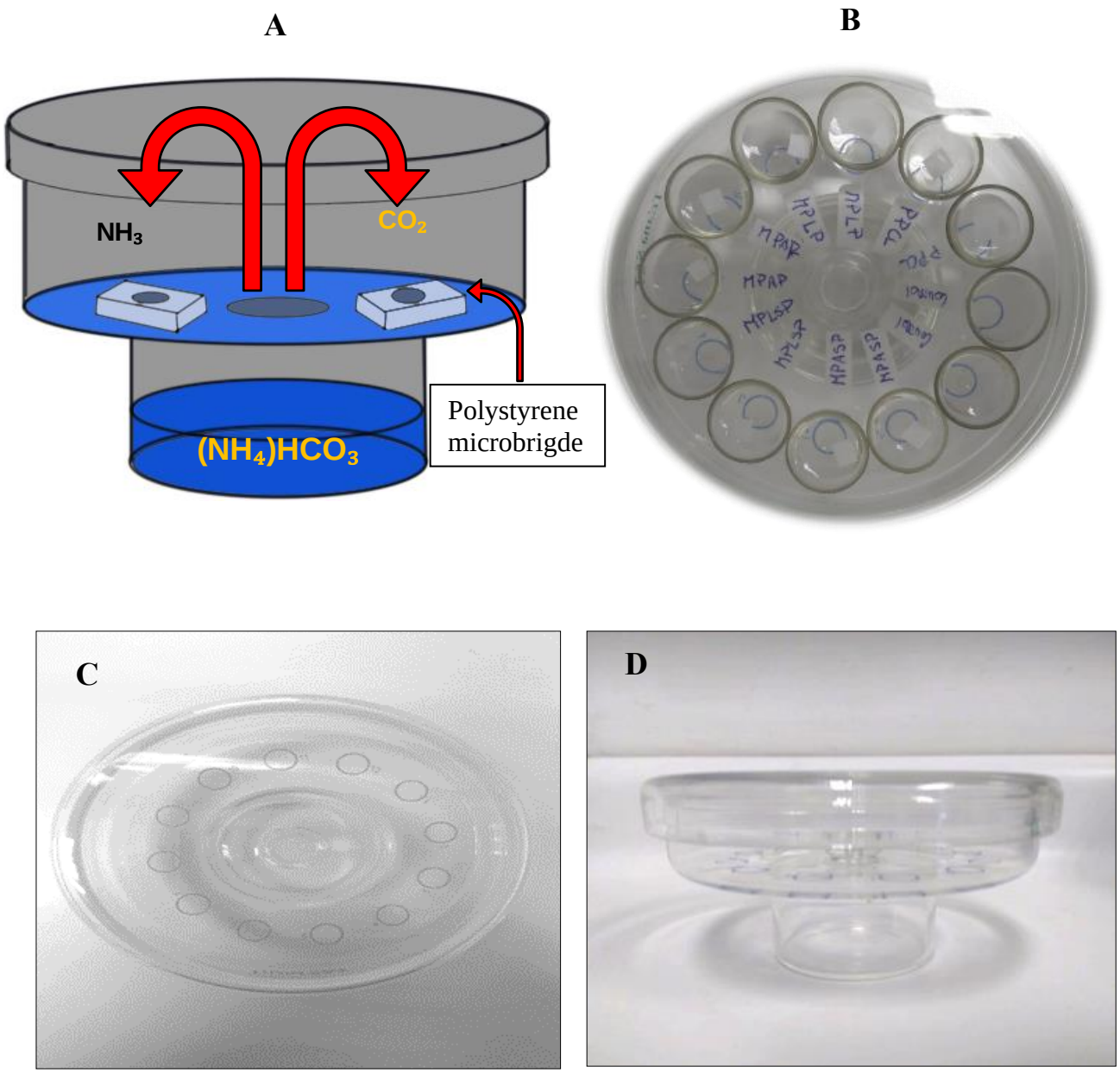

Figure S1. GD crystallization method: (A) experimental set up for $CaCO_3$ production, (B) subdivided crystallization chamber of 12 compartments containing a duplicate of each PCL samples as template, (C) top and (D) lateral views of GD crystallization camera.

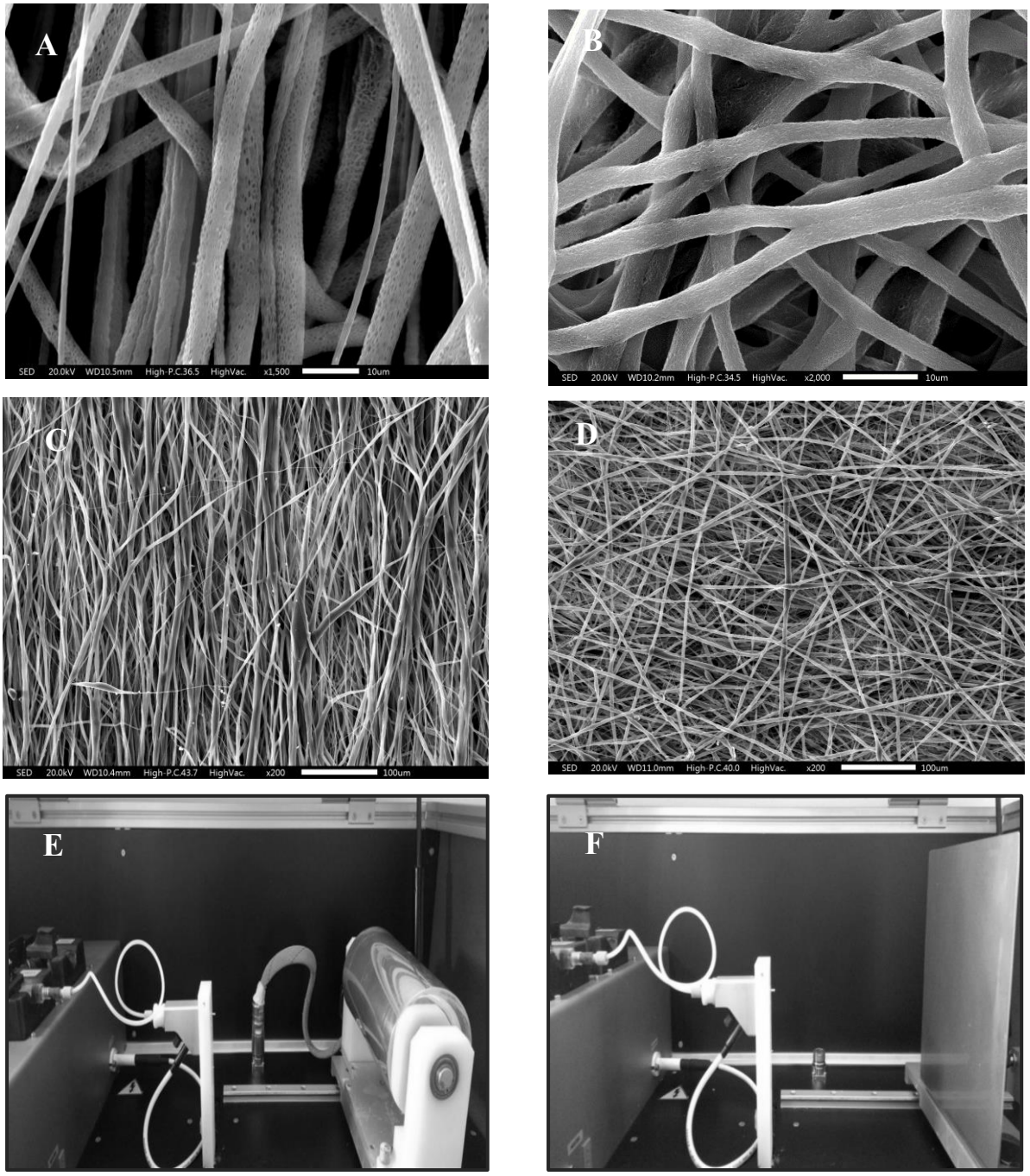

Figure S2. Aligned and random PCL fibers meshes obtained from 18% (w/v) PCL solution using ethyl acetate/acetate 3:1 (v:v) solution with flat (30×30 cm) or rotating (10 cm in diameter) collectors, respectively. For porous PCL fibers meshes, ethyl acetate /DMSO 1:9 (v:v) was used as solvents. (A) PA-PCL fibers, (B) PR-PCL fibers, (C) A-PCL fibers, (D) E-PCL fibers, (E) electrospinning using rotating collector and (F) electrospinning using flat collector.

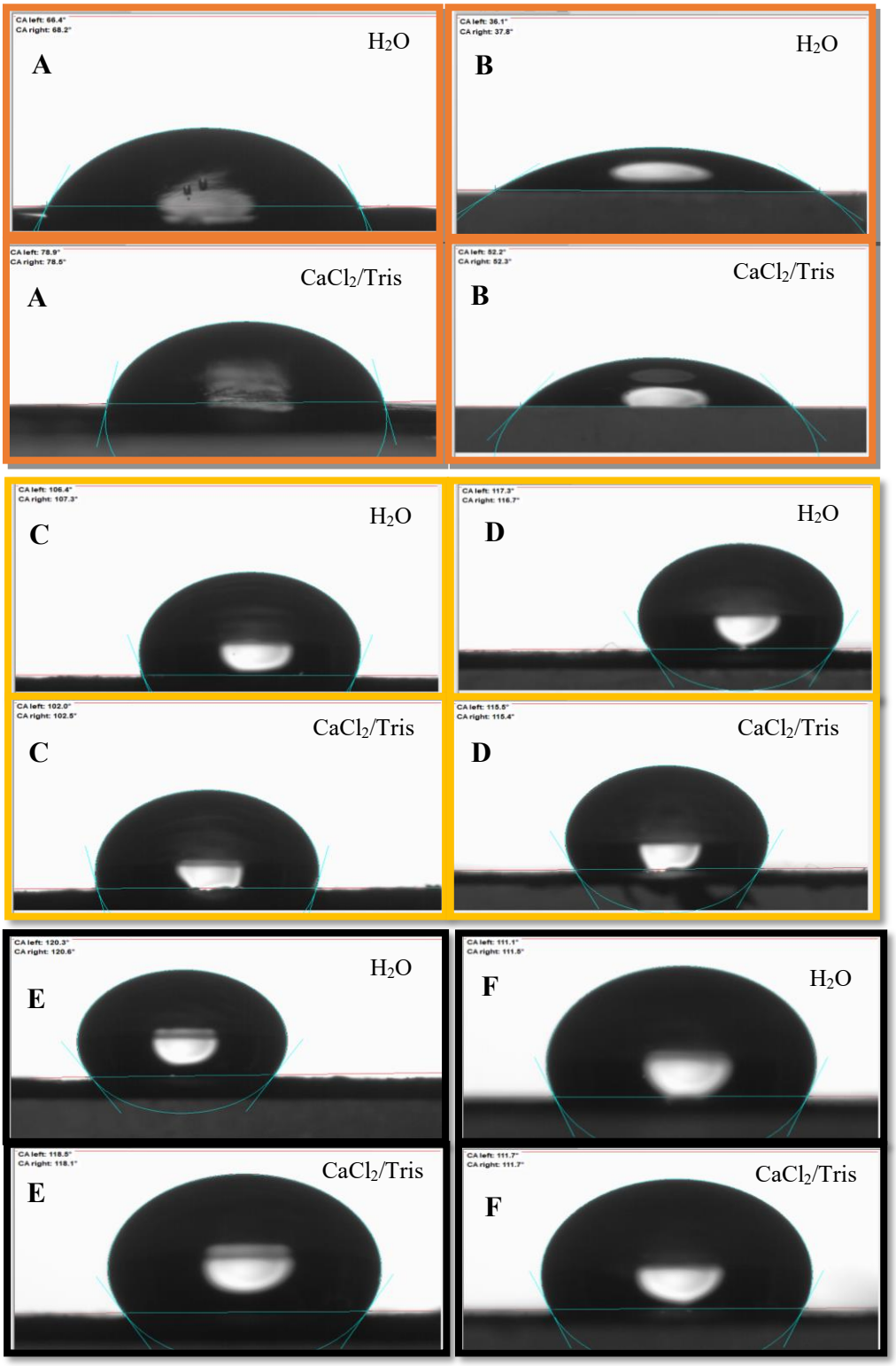

Figure S3. Contact angle [°] of water and $CaCl_2$/TRIS solution for melted PCL and glass used as controls, non- and porous- random and aligned electrospun PCL fiber meshes used as template in the *in vitro* GD crystallization of $CaCO_3$. (A) melted PCL film, (B) glass substrate, (C) NPR-PCL fiber mesh, (D) NPA-PCL fiber mesh, (E) PR-PCL fiber mesh and (F) PA-PCL fiber mesh.

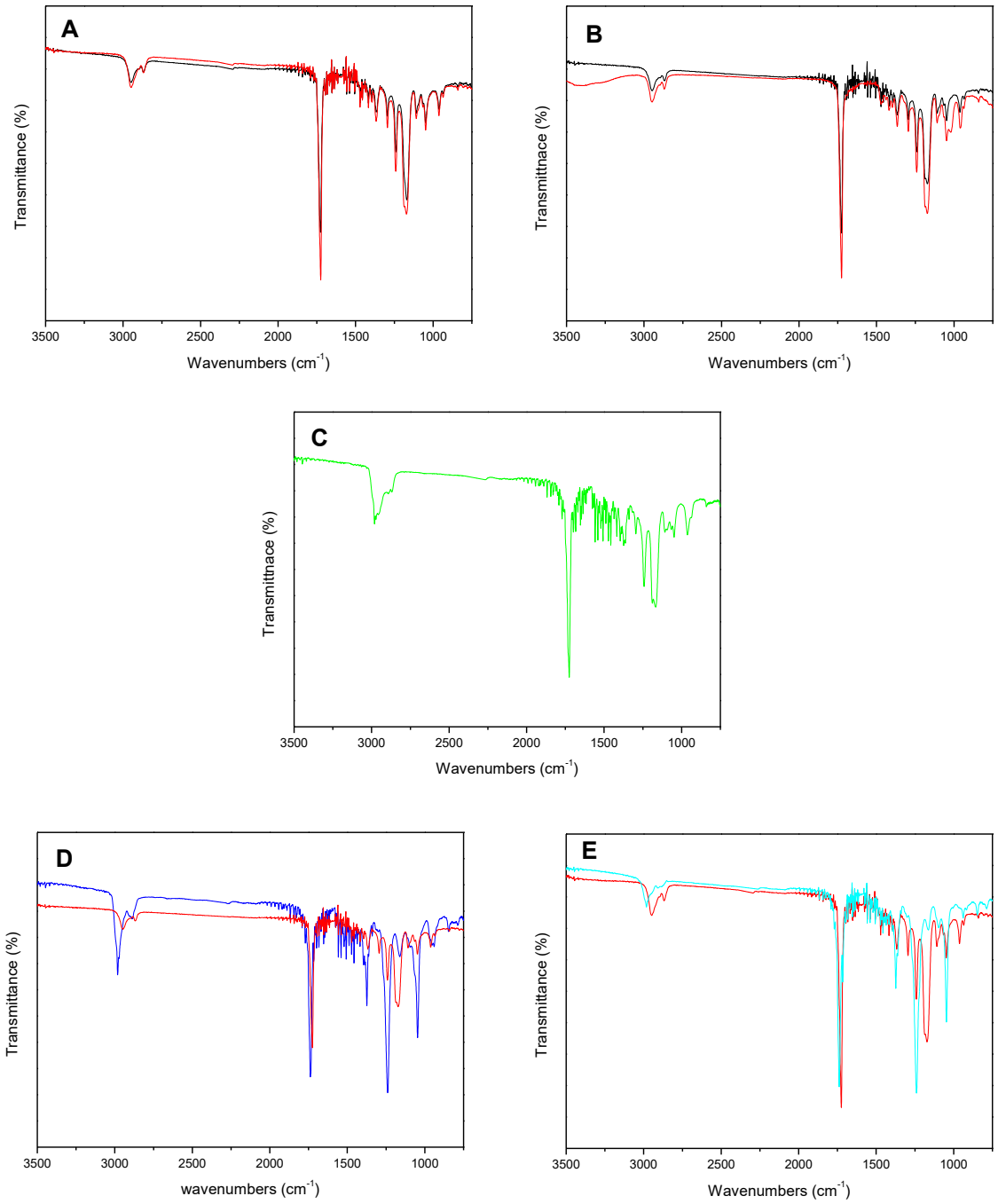

Figure S4. FTIR spectra of PCL samples. (A) non-porous random and aligned PCL fiber meshes, (B) porous random and aligned PCL fiber meshes, (C) melted PCL film, (D) PCL solution before electrospinning and porous random PCL fiber mesh, (E) PCL solution before electrospinning and non-porous random PCL fiber mesh. Red and black lines correspond to random and aligned PCL fibers. Blue and light blue lines correspond to 18% PCL solution in ethyl acetate/DMSO 1:9 (v:v) and ethyl acetate /acetone 3:1 (v:v) solutions, respectively.

2.2 Table S1

Table S1. Surface area summaries by MBET and BJH adsoprtion/desorption on different PCL substrates.

PCL substrate	BJH adsorption m^2/g	BJH desorption	MBET surface area
Control[2]	0.684	0.569	0.000
R-PCL mesh	0.745	3.201	0.000
A-PCL	1.514	1.743	0.000
PR-PCL mesh	3.497	5.560	0.660
PA-PCL mesh	5.307	12.101	0.003

Control [2] corresponds to melted PCL substrate.

3. References

(1) Neira-Carrillo, A.; Pai, R. K.; Fuenzalida, V. M.; Fernández, M. S.; Retuer, J.; Arias, J. L. Calcium carbonate growth modification by constituents releases from porous cellulose filter membranes. *J. Chil. Chem. Soc*. **2008**, 53, 1469-1473.

(2) Fernández, M. S.; Passalacqua, K.; Arias, J. I., Arias, J. L. Partial biomimetic reconstitution of avian eggshell formation. *J. Struct. Biol*. **2004**, 148, 1-10.