http://dx.doi.org/10.5012/bkcs.2012.33.7.2295

3D Micromorphology Producing within Poly(lactic acid) Skeleton Using Room-Temperature Ionic Liquids: From Particulate, Fibrous or Porous Scaffolds to Beads

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Received March 6, 2012, Accepted April 11, 2012

We describe herein a three-dimensionally diverse micropatterning of poly(lactic acid), as a biopolymer, using 1-butyl-3-methylimidazolium-based room-temperature ionic liquids (bmim-based RTILs), [bmim]X (X = SbF_6, PF_6, NTf_2, Cl). Utilizing the hydrophobic bmim-based RTILs, [bmim]X (X = SbF_6, PF_6, NTf_2) and a phase separation technique, we were able to produce white and opaque membranes with a three-dimensional structure closely packed with particles (10-50 μm in diameter). The particlulate structure, made by the assistance of [bmim]NTf_2 and DCM, interestingly transformed to a fibrous structure by using a cosolvent, *e.g.*, DCM/CF_3CH_2OH. When we used an increased amount of [bmim]NTf_2, the particles were effectively detached and macrosized (100-500 μm in diameter) and the oval-shaped beads were obtained in a powder form. By varying the counter-anion type of the imidazolium-based RTIL, for example from NTf_2^- to Cl^-, the particulate 3D-morphology was once more transformed to a porous structure. These reserch results could be potentially useful, as a method to fabricate particulate scaffolds, fibrous or porous scaffolds, and beads as a biopolymer device in diverse fields including drug delivery, tissue regeneration, and biomedical engineering.

Key Words : 3D micromorphology, Scaffold, Ionic liquid, Particle

Introduction

Two- and three-dimensionally (2 and 3D) micro- and macro-patterned polymeric skeletons have a wide range of applications in the following fields: chemistry, medical science, energy storage/conversion, and environmental engineering.[1] Particularly in the tissue engineering field, such micropatterns as micro-porous, -spherical and -fibrous geographies are intensively targeted to provide cells with a biomimic circumstance, in terms of cell-surface interaction.[2,3] Cell culture substrates with the various micropatterns have been fabricated by the conventional micropatterning methods, such as photolithography and various phase separation techniques. For the hard substrates like metal and glass, a photolithographic methods have been implemented for micropattnerning. Meanwhile, phase separation techniques were suitable for the micropatterning of a number of soft materials such as biopolymers and have been served as a general strategy, in order to obtain micro- and macro-patterned polymer skeletons. The phase separation techniques have been induced conventionally by several ways, including solvent-drying, cooling the polymer solution, changing the concentration ratio of components and *in situ* polymerization.[1-13] Among these, the solvent-drying method has been found to be the simplest and the most effective way.[4,7,11,13]

Ionic liquids (ILs) consist of bulky and asymmetric organic cations such as 1-alkyl-3-methylimidazolium, 1-alkylpyridinium, *N*-methyl-*N*-alkylpyrrolidinium and ammonium ions. They can bear a wide range of counteranions, for example, generally from simple halides such as Cl^- and Br^- to large inorganic and organic anions such as tetrafluoroborate (BF_4^-), hexafluoroantimonate (SbF_6^-), hexafluorophosphate (PF_6^-), bistriflimide (NTf_2^-), triflate (OTf^-). Ionic liquids (RTILs) having a melting point below 100 °C have been known as the room temperature ionic liquids (RTILs) and 1-butyl-3-methylimidazolium (bmim) based ionic liquids bearing anions *i.e.* SbF_6^-, PF_6^-, NTf_2^-, OTf^-, BF_4^-, Cl^- are typical RTILs. Some of them are in liquid form even at much lower temperatures, such as > −80 °C of [bmim]BF_4. RTILs have many fascinating properties.[14-18] For example, these liquids are highly polar and they can be made miscible and/or immiscible with water and a number of organic solvents. This is the case since, the type of anions of RTILs greatly affect their wettability, *i.e.*, hydrophilic/hydrophobic nature. The constituents of ILs are constrained by high coulombic forces, exhibiting practically no vapor pressure. Therefore, even at an elevated temerature, they are non-volatile. These unique traits of RTILs allow the possibility for more efficient applications, including polymer micropatterning by phase separation techniques.[19-24]

During our extensive efforts on the utilization of RTILs in various applications,[22-24] we reported that three-dimensionally micro-porous polymer membranes would be easily obtained by switching from an organic solvent to a hydrophilic ionic liquid.[25] Our study will aim to report the diverse effects on the 3D-morphologies, created inside and outside the produced membranes from the counter-anion of RTILs, the used amount of RTILs and the organic solvent effect. Indeed, the morphology was very diverse and changeable

from the interconnected particulate structures to the interconnected fibrous structures, to porous honeycomb structures, and finally, to the disconnected oval-shaped beads. These all are very useful morphologies for the devices used in diverse fields, including drug delivery, tissue regeneration, and biomedical engineering.

Experimental

The ILs, [bmim]X (bmim = 1-butyl-3-methylimidazolium cation; X = SbF_6, PF_6, NTf_2, OTf, BF_4 and Cl), were purchased from C-Tri Co., Ltd. (Korea, www.c-tri.co.kr) and were used without further purification. Polylactic acid (PLA) was purchased from Boehringer Ingelheim.

Poly(lactic acid) (PLA, from Boehringer Ingelheim) was used as the biodegradable model polymer. The ILs, [bmim]-X (bmim = 1-butyl-3-methylimidazolium cation; X = SbF_6, PF_6, NTf_2, and Cl; purchased from C-Tri Co., Ltd, Korea) as a room temperature ionic liquid and CH_2Cl_2 (DCM) as an organic solvent, were chosen. The materials were used without further purification. The micro-patterned PLA-membranes were prepared according to the following procedures. Briefly, 0.2 g of PLA, 1.0 g of an ionic liquid and 10 mL of DCM were mixed to produce a transparent solution. The organic solvent (DCM) was then evaporated from the transparent solution, under ambient conditions. After which, rubbery white gels remained at the bottom of the glass dish. To remove the RTILs, the rubbery films obtained were soaked with shaking in ethanol (or water) for about 3 h. The samples were then dried under ambient conditions, which gave white and opaque membranes. The ionic liquid recovered was reused for the next run.

Morphology of the 3D structured PLA scaffolds and their physicochemical properties were examined with the scanning electron microscopy (SEM; JEOL and HITACHI S-3000H, Japan) and FT-IR spectrometer (JASCO 470 PLUS), respectively. For scanning electron microscopic analysis, the samples were sputter-coated with approximately 10 nm of gold, before analysis. The infrared spectra were recorded in solid condition at a range of 400-4000 cm^{-1}. Thermogravimetric analysis (TGA) was conducted using a Seiko Exstar 6000 TG/DTA6100 (SEICO INST., JAPAN) with a heating rate of 10 °C/min at temperatures ranging from 20 to 550 ºC under air.

Results and Discussion

Polylactic acid (PLA) as a biodegradable polymer and 1-butyl-3-methylimidazolium-based ionic liquids bearing different counteranions ([bmim]X; X = SbF_6, PF_6, NTf_2, and Cl) were chosen as the model substrates. Chemical structures of used RTILs and photographs of polymer/RTIL/solvent solution and as-prepared polymer membrane are shown in Figure 1.

To prepare the 3D-micropatterned polymer network, PLA and one of a series of ionic liquids were homogeneously dissolved in dichloromethane (DCM), which led to the formation of a viscous solution. Upon evaporation of the casting solution at room temperature, a transition from a homogeneous, fluent liquid state to a gel state was observed.

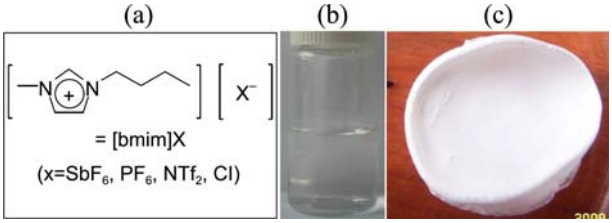

Figure 1. (a) Chemical structure of RTILs ([bmim]X = 1-butyl-3-methylimidazolium-based ILs (X = SbF_6, PF_6, NTf_2, and Cl)) and (b) and (c) photographs of polymer/RTIL/solvent solution and as-prepared polymer membrane after solvent-drying and RTIL removal.

Upon selective extracting of the RTIL and drying from the polymer gel with ethanol (or water), white opaque membranes in the solid state were obtained (Figure 1). The recovered ionic liquids could be reused for the following runs without any loss of their physicochemical properties.

SEM images of the membranes produced by using RTILs bearing anions, such as SbF_6, PF_6 and NTf_2, interestingly showed a three-dimensional particulate structure (Figure 2). Particles resulted from [bmim]SbF_6 and [bmim]PF_6 were compactly packed with a very fine grain size (10-20 μm) and with clear boundary. However, membraine obtained by utilizing [bmim]NTf_2 exhibited a larger particle size (30-50 μm) and some loosely packed structure, resulting from relatively less hydrophobicity of the RTIL. Infrared spectroscopy showed the most common feature of the polyester, including the C=O absorption occurring at 1749 cm^{-1} and the C-O absorptions occurring at about 1183 and 1081 cm^{-1}.

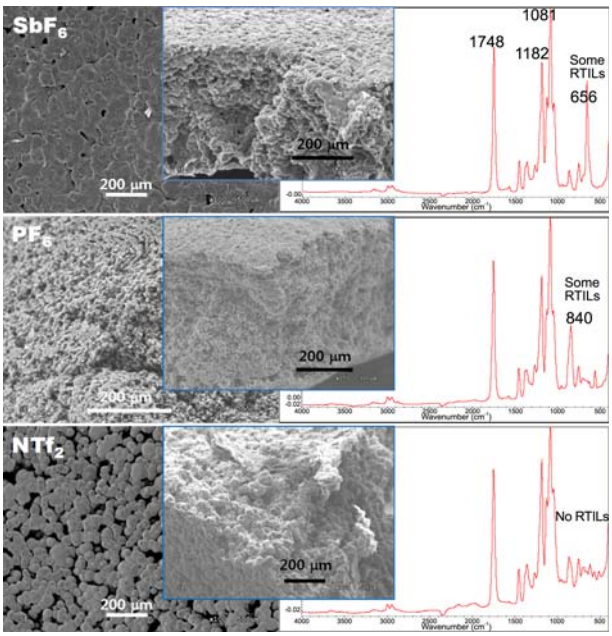

Figure 2. Scanning electron micrographs and FT-IR spectra of 3D-micropatterned PLA membranes prepared by using [bmim]X (X = SbF_6, PF_6 and NTf_2).

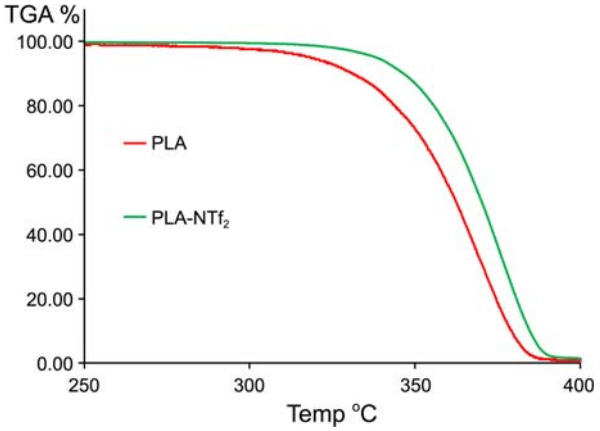

Figure 3. Termogram of the pattern-free film (PLA) and the membrane (PLA-NTf_2) micropatterned using [bmim]NTf_2.

Moreover, the spectra of PLA-SbF_6 and -PF_6, unlike PLA-NTf_2, showed the halogen-related peaks at < 1000 cm^{-1} (Figure 2). This observation suggests that a bit of ionic liquids still remained to be isolated between the grains, even after several extractions because of the close packing of granules. Mechanistically, the particulate 3D-morphology may originate from the difference in the wettability of the RTIL and the polymer used. During drying of the organic solvent, the ionic liquid phase might be slowly separated from the polymer phase, to form a RTIL-interpenetrated polymer gel. When the ionic liquid used has a greater hydrophobic tendancy than the PLA, the relatively hydrophilic polymer phase may be forced to solidify into a spherical form to minimize its own surface area. However, the ionic liquid phase will mostly be present outside of the solid polymer phase. After complete removal of the ionic liquid, surrounding the polymer particles, the three-dimensionally interconnected microspheres will come into view as a large skeleton. Upon thermogravimetric analysis (TGA) (Figure 3), PLA membranes 3D-micropatterned by [bmim]NTf_2 were found to decompose at temperatures ranging from 300 to 390 °C, without secondary decomposition. However, the pattern-free PLA film without any pattern started to thermaly degradate at about 250 °C. It is possible that granules with a higher density caused the delay of the initiation time to degradate.

We newly introduced the TFE with higher hydrophilicity, as an organic cosolvent of DCM (5 mL TFE/5 mL DCM), to improve the penetrability of the ionic liquid into the polymer during drying process. TFE is a protic polar solvent, while DCM is an aprotic polar solvent. In this case, the higher penetrability of TFE could be expected from it's strong intermolecular interactions with the polymer molecules, for example, *via* polar-polar interactions and additional hydrogen-bondings. As a result, an effective fenetration of ionic liquids, which are dissolved in the solvent system, are responsible for the tearing of the interconnected pieces (or fiber) of the polymer particles.

The formation of the three-dimensionally fibrous morphology could be expected after removal of the solvents and the

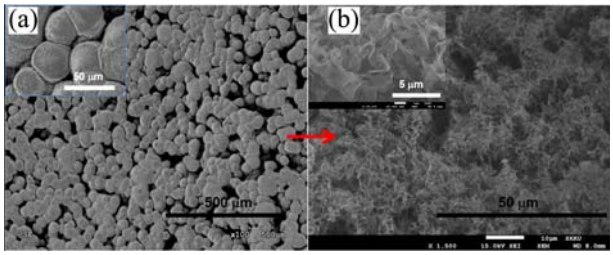

Figure 4. The effect of organic solvent: SEM images of the PLA scaffolds produced by using (a) [bmim]NTf_2/CH_2Cl_2 and (b) [bmim]NTf_2/CH_2Cl_2/CF_3CH_2OH.

RTIL. Indeed, the SEM images in Figure 4 clearly illustrates the expected morphological transition from the particulate morphology to a fibrous morphology. Fibrous pieces were interconnected three-dimensionally and distributed throughout the polymer. This method could be of potential use to make the PLA membranes with fibrous morpology as a tissue regeneration scaffold. This is so since the presence of the pore space and the fibrous structure facilitates the supply of nutrients of the cells and the ingrowth of neo-tissues. Studies that warrant the biological performance of the newly-developed particulate and fibrous scaffolds are in progress.

Micro- or macro-sized scaffold particles are needed to provide the cells with structural support or to deliver the cells and growth factors, particulally for cell-replacement therapy and tissue regeneration using stem cells. To know if the increment of the RTIL amount can actually cause the individual separation of closely packed granules and the increment of the individually separated granule size, we conducted the same experiment in the presence of an excess amount of RTIL (0.2 g PLA, 1 mL DCM and 2 mL [bmim]NTf_2). After air drying of the casting solution at room temperature, a gel formation was also observed. However, during extracting RTIL from the gel, using ethanol (or water), the product was acquired as a white opaque powder. The SEM image revealed the effective breakup into particles and the size increments of the obtained individual granules of up to 500 mm in diameter (Figure 5). In the solution, the relatively hydrophilic PLA particulate phases could be fully surrounded by the relatively hydrophobic ionic liquid phases, due to the presence of excess amount of the RTILs and several polymeric particulate phases. Which then could

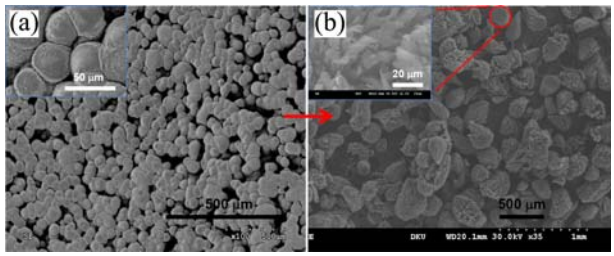

Figure 5. The relative amount effect of RTIL: SEM images of (a) paticulates connected with one another as a scaffold (PLA/[bmim]-NTf_2 = 0.2 g/1 g) and (b) separatly detached beads as powder (PLA/[bmim]NTf_2 = 0.2 g/1.5 g).

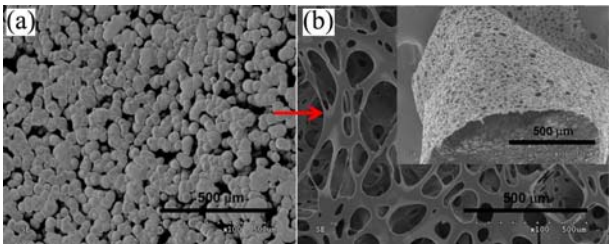

Figure 6. The hydrophilicity effect of RTIL: SEM images of (a) the paticulate structure in a scaffold (PLA/[bmim]NTf_2) and (b) the porous morphology in a scaffold (PLA/[bmim]Cl) with inset showing a tublar, porous and thin membrane.

be mersed into a larger particulate phases to minimize the interfacial tension between both phases that leads to an effective disconnection and the effective growth of the polymer particles after the isolation of the product amterial as a powder form. Interestingly, the as-prepared oval-shaped beads have fibrous surface morphology. These morphological charateristics could make possible an effective application of these particles, as particle scaffolds to support and to deliver the cells and growth factors.

Finally, the wettability change of RTILs interestingly led to the change of the pattern shape. For example, as we previously reported, the use of imidazolium-based hydrophilic RTILs bearing anions, such as OTf, BF_4 and Cl, created a highly porous structure instead of the particulate structure.[25] SEM images in Figure 6 clearly showed the sharp contrast of the geometrical patterns three-dimensionally created by using of the [bmim]NTf_2 and [bmim]Cl. The morphological inversion could be understood in terms of the formation of relatively hydrophilic ionic liquid droplets and a relatively hydrophobic polymer matrix. This phenomenon could be applied for the preparation of the tublar, porous and thin membrane, as shown in the inset of Figure 6.

In conclusion, we prepared herein PLA membranes with diverse morphologies, for example, particulate structure, fibrous or porous structures, and beads using 1-butyl-3-methylimidazolium-based room-temperature ionic liquids (bmim-based RTILs), [bmim]X (X = SbF_6, PF_6, NTf_2, Cl). The interesting morphological transformations were induced by the diverse changes, for example, the counter-anion of RTILs, the used amount of RTILs and the organic solvent. These reserch results could be potentially useful as a methodology to fabricate particulate scaffolds, fibrous or porous scaffolds, and beads as a biopolymer device in the use of diverse fields including drug delivery, tissue regeneration, and biomedical engineering.

Acknowledgments. This study was supported by the research fund of Dankook University in 2011.

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