

METHOD FOR PREPARING BIOCOMPATIBLE SCAFFOLD AND SCAFFOLD PREPARED THEREFROM

BACKGROUND OF THE INVENTION

Field of the Invention

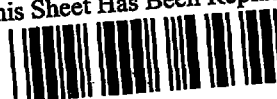
The present invention relates to a method for preparing a biodegradable and biocompatible, porous, polymeric scaffold which can serve as support or matrix for cell or tissue culture. More particularly, the present invention relates to the method preparing a three-dimensional porous, polymeric scaffold with better biocompatibility characterized by adopting a effervescent salt and a biocompatible scaffold prepared therefrom.

Description of the Related Art

To be used for bio-tissue culture, polymers are basically required to be of biocompatibility and biodegradability. The aliphatic polyesters which bear lactic acid or glycolic acid as a backbone unit were approved as being satisfactory to the requirement by the Food and Drug Administration (FDA), U. S. A., and most widely used now. Examples of such biocompatible and biodegradable aliphatic polyesters include poly (lactic acid) (PLA) poly (glycolic acid) (PGA), poly (D, L-lactic-co-glycolic acid) (PLGA), poly (caprolactone), poly (valerolactone), poly (hydroxybutyrate), poly (hydroxy valerate), etc.

Proven to be biocompatible, the aliphatic polyesters have been widely used as drug delivery carriers or sutures for a long period of time.

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PLGA is found to afford biodegradable polymers with various degradation periods by controlling the ratio of lactic acid monomer and glycolic acid monomer and/or modifying the synthesis procedure thereof.

In addition to biodegradability and biocompatibility, other requirements for the polymers for bio-tissue culture are a surface area large enough to allow cell adhesion at high densities, a pore size large enough to enable the vascularization in the cultured tissue after transplantation into a host and the transmission of substances, such as nutrients, growth factors and hormones; and the interconnectivity of the pores.

Typically, the porous polymeric scaffolds fulfilling the above requirements are prepared as follows.

The most popular and commercially available are scaffolds consisting of PGA sutures (unwoven PGA fiber mesh). They are made in three-dimensional shapes by thermally treating randomly entangled threads of suture.

The mesh exhibits very high porosity and sufficiently large pore size in addition to being of high interconnectivity, but finds a limited range of applications on account of poor mechanical strength (A. G. Mikos, Y. Bao, L. G. Cima, D. E. Ingber, J. P. Vacanti, and R. Langer, *J. Biomed. Mater. Res.* (1993) 27,183-189).

Another preparing method of the porous polymeric scaffolds is of particulate leaching, favored by A. G. Mikos et al. (A. G. Mikos, G. Sarakinos, S. M. Leite, J. P. Vacanti, and R. Langer, *Biomaterials* (1993) 14, 5,323-330; A. G. Mikos, A. J. Thorsen, L. A. Czerwonka, Y. Bao, R. Langer, D. N. Winslow, and J. P. Vacanti, *Polymer* (1994) 35,5,1068-1077). The particulate leaching method has an advantage of easily controlling pore sizes of the scaffolds in dependence on



the size of the salt (NaCl) employed, but suffers from a disadvantage in that salts remaining in the scaffolds or their rough morphology cause cell damage.

Besides, an emulsion freeze-drying method and a high pressure gas expansion method can be used for the preparation of such scaffolds (K. Whang, C. H. Thomas, K. E. Healy, G. Nuber, *Polymer* (1995) 36,4,837-842; D. J. Mooney, D. F. Baldwin, N. P. Suh, J. P. Vacanti, and R. Langer, *Biomaterials* (1996) 17,1417-1422). Despite their own advantages, the methods have the limitation of there being difficulties in making open cellular pores.

In recent, attempts have been made to construct the scaffolds by taking advantage of the phase separation of polymer solutions (H. Lo, M. S. Ponticiello, K. W. Leong, *Tissue Eng.* (1995) 1,15-28; H. Lo, S. Kadiyala, S. e. Guggino, K. W. Leong, *J. Biomed. Mater. Res.* (1996) 30,475-484; Ch.Schugens, V. Maguet., Ch. Grandfils, R. Jerome, Ph. Teyssie, *J. Biomed.Mater. Res.* (1996) 30,449-461).

As mentioned above, various methods have been developed for the preparation of three-dimensional polymeric scaffolds in which cell adhesion and differentiation can be induced. Nevertheless, there remain problems to be solved in preparing three-dimensional scaffolds for tissue culture with biodegradable polymers. At present, only a few companies, such as Advanced Tissue Science Inc. and Texas Biotechnology Inc. have been successful in the commercialization of such scaffolds, wherein PGA suture is utilized on a small scale.

SUMMARY OF THE INVENTION

To overcome the above mentioned shortcomings, the inventor et al. have made intensive studies and as a result developed a method for preparing

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biodegradable, three-dimensional, porous scaffolds for tissue culture, which , thereby the resulting scaffolds can be molded in desirable shapes and have desirable pore size and porosity.

Accordingly, an object of this invention is to provide a method for preparing biodegradable, three-dimensional, porous scaffolds with improved biocompatibility.

Another object of this invention is to provide scaffolds for tissue culture with various pore size and porosity.

The present invention pertains to the method for preparing biodegradable, three-dimensional, porous scaffolds comprising the steps of (i) dissolving a polymer in an organic solvent to prepare a polymeric solution; (ii) mixing an effervescent salt in the resulting polymeric solution to give a polymer/salt/organic solvent mixed gel; (iii) removing the organic solvent from the polymer/salt/organic solvent mixed gel; and (iv) immersing the gel in acidic solution to render the salt to effervesce to yield a polymeric scaffold.

Alternatively, the present method for preparing biodegradable, three-dimensional, porous scaffolds comprises the steps of (i) dissolving a polymer in an organic solvent to prepare a polymeric solution; (ii) adding a nonsolvent to prepare the resulting polymeric solution such that the polymer is precipitated and concentrated to form a polymeric gel; (iii) mixing an effervescent salt in the polymeric gel to give a polymer/salt/organic solvent mixed gel; (iv) removing the organic solvent from the polymer/salt/organic solvent mixed gel; and (v) immersing the gel in acidic solution to render to the salt to effervesce to yield a



polymeric scaffold.

Moreover, the present invention pertains to a porous polymeric scaffold for tissue engineering characterized in that a porosity and a pore size of the scaffold are varied depending on a concentration of acidic solution, and a particle size and an amount of effervescent salt used in process for preparing the scaffold.

BRIEF DESCRIPTION OF THE DRAWINGS

The above and other objects, features and other advantages of the present invention will be more clearly understood from the following detailed description taken in conjunction with the accompanying drawings, in which:

Fig. 1 is a SEM photograph showing the magnified surface of the poly (D, L-lactic-co-glycolic acid)-based, porous scaffold, prepared in Example I

Fig. 2a is a SEM photograph showing the surface of the poly (D, L-lactic-co-glycolic acid)-based, porous scaffold with a thickness of 2 mm and a diameter of 10 mm, prepared in Example II;

Fig. 2b is a magnified photograph of Fig. 2a;

Fig. 2c is a SEM photograph showing the surface of the poly (D, L-lactic-co-glycolic acid) based, porous scaffold with a thickness of 5 mm and a diameter of 10 mm, prepared in Example II;

Fig. 2d is a magnified photograph of Fig. 2c;

Fig. 3a is a SEM photograph showing the cross section of the poly (D, L-lactic-co-glycolic acid)-based, porous scaffold with a thickness of 2 mm and a diameter of 10 mm, prepared in Example II;

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Fig. 3b is a magnified photograph of Fig. 3a;

Fig. 3c is a SEM photograph showing the cross section of the poly (D, L-lactic-co-glycolic acid)-based, porous scaffold with a thickness of and a diameter of 10 mm;

Fig. 3d is a magnified photograph of Fig. 3c;

Fig. 4 is a SEM photograph showing the rat hepatocytes which have been inoculated on the porous scaffold of Fig. 1 and cultured for 7 days; and

Fig. 5 is an optical microphotograph showing the distribution of viable hepatocytes after an MTT assay for the determination of viability of cultured cells.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

Before the present method and scaffold are disclosed or described, it is to be understood that the terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting. It must be noted that, as used in the specification and the appended claims, the singular forms "a", "an" and "the" include plural referents unless the context clearly dictates otherwise.

Throughout this specification and claims, where publication are referenced, the disclosures of these publications in their entireties are hereby incorporated by reference into this application in order to more fully describe the state of the art to which this invention pertains.

In the present invention, the preparation of biodegradable and biocompatible, three-dimensional, porous scaffolds for tissue culture is based

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on phase separation and particulate leaching. First, a polymer is dissolved in an organic solvent. Preferably the resulting polymeric solution is highly concentrated solution with high viscosity.

The polymer employed in this invention is preferably a biodegradable and biocompatible polymer in view of the object of this invention. The polymer is preferably polyester-based polymer, more preferably a aliphatic polyester-based polymer, and the most preferably is one selected from the group consisting of poly (L-lactic acid) (PLLA), amorphous poly (D, L-lactic acid) (PDLLA), poly (glycolic acid), poly (D, L-lactic-co-glycolic acid) (PLGA), poly (caprolactone), poly (hydroxy butyrate), poly (dioxanone) and copolymers of these polymers.

The polymer may be used irrespective of molecular weight, but better results are obtained from those whose molecular weight is in the range of 5,000-500,000.

Examples of the organic solvent for use in dissolving the polymers include, but not limited to, methylene chloride, chloroform, acetone, dimethylsulfoxide, dimethylformamide, N-methylpyrrolidone, dioxane, tetrahydrofuran, ethylacetate, methylethylketone, and acetonitrile.

Optionally, the polymer solution is further mixed with a nonsolvent so as to concentrate the solution into a gel phase of a concentrated solution. It is preferred that the nonsolvent used in the alternative method substantially undissolves the polymer. Non-limiting example of the nonsolvent includes ethanol, methanol, aqueous ethanol, isopropyl alcohol, diethyl ether, hexane, heptane and petroleum ether.

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Through the above step, it is possible to prepare porous polymeric scaffolds even with biodegradable, low-molecular weight polymers which cannot be conventionally used as materials on account that their solutions are of low viscosity even at high concentrations.

Then, the polymeric solution is homogeneously mixed with an effervescent salt, followed by the removal of the organic solvent from the resulting polymer/salt/organic solvent mixed gel. Immersing the organic solvent-free polymeric/salt gel slurry in acidic solution allows the salt to effervesce, resulting in a porous structure.

With a size of 100-500 μm , the effervescent salt is selected from the group consisting of ammonium carbonate, ammonium bicarbonate, sodium carbonate, and sodium bicarbonate. It is preferred to use the salt at such an amount that the weight ratio of the polymer to the effervescent salt may be in the range from 1:1 to 1:100.

Depending on the organic solvent remaining in the polymer/salt/organic solvent mixed gel, various methods may be utilized to remove the organic solvent. Organic solvents with relatively low boiling points, such as methylene chloride, chloroform and dioxane, are removed through drying at atmosphere pressure or under vacuum whereas in case of that high-boiling point solvent such as dimethylsulfoxide and methylpyrrolidone is employed, the solvent can be removed by drying at atmospheric pressure or under vacuum following replacement with low boiling point solvents such as ethanol and methanol.



According to this invention, the acidic solution enables the salt to effervesce at relatively lower temperature, for example at room temperature, within a short period of time. In addition, its concentration affects the size of the pores formed in the scaffolds, so that the pore size can be under the control of the concentration. Therefore, the effervescence of the salt with the acidic solution makes it possible to prevent the thermal distortion of the polymer and to form pores at desirable sizes as well as to settle down the drugs inside the porous scaffold for cell culture, if necessary.

Preferably, the acidic solution is a solution of one selected from the group consisting of citric acid, hydrochloric acid, acetic acid, formic acid, tartaric acid, salicylic acid, benzoic acid and glutamic acid. For use, the acid is dissolved to the concentration of 1 % or supersaturation in water or in an aqueous solution saturated with an organic solvent such as methylene chloride, chloroform, dioxane, dimethylsulfoxide and methyl pyrrolidone.

To yield a practical scaffold, it is preferred that the resulting polymeric scaffold is washed with unreactive solution such as distilled water and is then dried by conventional drying method such as freeze-drying, heat drying and vacuum drying.

The porous scaffold of this invention is characterized in that its porosity and pore size can be adjusted by varying a concentration of acidic solution, and a particle size and an amount of effervescent salt used in the above process.

Preferably, the porous scaffold of this invention is prepared in the above

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processes.

A better understanding of the present invention may be obtained in light of the following examples which are set forth to illustrate, but are not to be construed to limit the present invention.

EXAMPLE I: Preparation of Porous Scaffold from Poly (D, L-Lactic-co-Glycolic Acid) with Polymeric Solution

In chloroform, poly (D, L-lactic-co-glycolic acid) (PLGA) 65/35 with a weight average molecular weight of 180,000 was dissolved at an amount of 30% by weight. To the resulting polymeric solution of high viscosity, ammonium bicarbonate particles ranging, in size, from 180 to 300 μm , μm were added at weight ratios of 1: 10, 1: 15 and 1: 20 polymer: salt, respectively, followed by homogeneously mixing to yield polymer/salt/solvent gels.

After being introduced into a Teflon mold which was 2 mm thick with a diameter of 5 mm, the gels each were deprived of the solvent methylene chloride by evaporation at the atmospheric pressure. Each of the polymer/salt mixtures separated from the mold was mixed to 3 liters of citric acid solutions of various concentrations (20%, 40%, 60% and supersaturated), and stirred to effervesce the salt. After completion of the effervescence, the porous polymeric scaffolds thus prepared were drawn off, washed with distilled water and dried in a vacuum drier.

With the aid of a mercury intrusion porosimetry (Porous Materials Inc., Ithaca, NY), the scaffolds were measured for porosity and total pore volume, and the results are summarized in Table 1, below:

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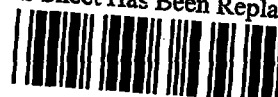


TABLE 1

Porosity and Pore Volume of the Resulting Polymeric Scaffold

Citric Acid Conc.	Pore Diameter (μm)	Pore Volume (cc/g)	Porosity (%)
20	122.03 ± 22.56	8.0603	98.03
40	142.49 ± 36.24	8.396	98.04
60	163.44 ± 0.74	9.2803	98.11
Supersaturated	186.24 ± 22.86	9.9842	98.64

An observation was made of the whole figures, and surface and cross section structures of the scaffolds, and the configuration of their inner pores through scanning electron microscopy (SEM) (Phillips 535M) and the results are given in Fig. 1. Before the observation, the polymeric scaffolds were coated with gold in an argon atmosphere at 5 psi for 5 min under an electric field of 5 mA by using a sputter (Hummers, techniques U. S. A.).

A measurement was also made of the compression of modulus of the porous polymeric scaffolds prepared above. In this regard, the Instron 5538 was used to descend a load cell of 10 newton (N) at a speed of 2 mm/min vertically on a scaffold specimen, which was of a cylindrical shape 12 mm high with a diameter of 6 mm according to ASTM F451-95. The results are given, along with the porosity, in Table 2, below.

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TABLE 2

Porosity and Compression of Modulus of the Resulting Polymeric Scaffold

Salt: Polymer (weight ratio)	Porosity (%)	Compression of Modulus(kPa)
10:1	98.64	29.24 ± 0.40
15:1	98.92	16.45 ± 7.75
20:1	99.11	11.91 ± 0.54

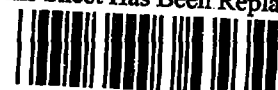
As demonstrated in Table 1, higher concentrations of citric acid cause the salt to undergo more active effervescent reaction, resulting in greater increase in pore size and porosity. In addition, it is also recognized from the data of Table 2 that increasing the salt ratio to the polymer results in increasing the porosity while reducing the compression of modulus of the polymeric scaffold. That is, an increase in the porosity leads to a reduction in the compression of modulus of the porous scaffold.

EXAMPLE II: Preparation of Porous Scaffold from Poly (D, L-Lactic-co Glycolic Acid) through Polymer Precipitation

A solution of poly (D, L-lactic-co-glycolic acid) (PLGA) 65/35, as used in Example I, in chloroform was added with an excess of ethanol and allowed to stand for 10 min to precipitate the polymer. After concentration, the polymeric precipitate maintained itself in a gel phase.

To the polymeric precipitate free of ethanol, ammonium bicarbonate particles ranging, in size, from 180 to 300 μm were added at a mass ratio of 1: 10 polymer: salt. The polymer/salt/solvent gel slurry prepared contained an even less amount of organic solvent than did that of Example I.

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After being introduced into two Teflon molds which were 2 mm and 5 mm in thickness with a diameter of 5 mm, respectively, the gel was deprived of the solvent by evaporation at the atmospheric pressure. The polymer/salt mixtures separated from the molds were mixed to 3 liters of supersaturated citric acid solution and stirred to effervesce the salt. After completion of the effervescence, the porous polymeric scaffolds thus prepared were drawn off, washed with distilled water and dried in a vacuum drier.

An observation was made of the whole figures, and surface and cross section structures of the scaffolds, and the configuration of their inner pores through a scanning electron microscope, as in Example I and the results are given in Figs. 2 and 3. As shown in these figures, the porous scaffolds prepared according to the invention have pores of uniform sizes superior in interconnectivity and uniformly distributed over the themselves regardless of the pore size.

EXAMPLE III

Porous polymeric scaffolds were prepared in similar manners to that of Example I, except that PLGA 50/50 and PLGA 75/25 were used instead of the biodegradable polymer PLGA 65/35. With the aid of a mercury intrusion porosimetry, the polymeric scaffolds were measured for porosity, pore diameter and surface area and the results are given in Table 3, below.

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TABLE 3

Pore Diameter, Porosity and Surface Area of the Resulting Polymeric Scaffold

Scaffold	Pore Diameter (μm)	Porosity (%)	Surface Area (m^2/g)
PLGA 50: 50 (3mm thick)	121.59	86.60	89.21
PLGA 65: 35 (3mm thick)	206.4	89.21	89.89
PLGA 65: 35 (5mm thick)	210.51	88.73	91.96
PLGA 75: 25 (3mm thick)	199.27	89.89	93.49
PLGA 75: 25 (5mm thick)	208.71	91.96	91.15

Apparent from the results of Table 3 is that no great differences in porosity and total pore volume exist between porous polymeric scaffolds prepared through polymer precipitation and polymer solution.

The process described in Example II has an advantage over that of Example I in that even smaller amounts of organic solvents are contained in the polymer/salt/organic solvent gels and thus, can be more readily removed, thereby allowing various drugs to be incorporated effectively therein, if necessary.

TEST EXAMPLE I: Cell Culture Using Poly (D, L-Lactic-co-Glycolic Acid)

Porous Scaffold

To confirm the suitability to three-dimensional cell culture of the porous, polymeric scaffolds prepared, rat hepatocytes were transplanted into the porous, polymeric scaffold according to a well-known technique (P.

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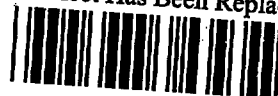
M. Kaufmann, et al., Cell Transplantation (1997) 6,5,463-468) and cultured for 7 days (Fig. 4). The number of the hepatocytes to be transplanted was in the range of 7×10^4 to 8×10^4 per porous scaffold. As many hepatocytes as in this range were found to be about 90-95% in transplantation efficiency. This was believed to result from the uniform distribution of the introduced hepatocytes over the porous scaffolds which were superior in the interconnectivity among the pores. The porous polymeric scaffolds into which the hepatocytes were transplanted were incubated for 7 days at 37°C in the presence of 5% CO_2 in an incubator to examine the viability of the cells. In this regard, an MTT (3-(4,5-dimethylthiazol-2-yl)-2,4 diphenyltetrazolium bromide) assay was conducted. As shown in Fig. 5, viable cells were uniformly distributed over the whole scaffold structure.

In Table 4, below, cell viability for 7 days of incubation is given, along with secreted albumin amount, an indicator for the differentiating function of hepatocytes.

TABLE 4

Cell Viability and Secreted Albumin Amount of Hepatocytes Cultured in Porous Polymeric Scaffolds for 7 Days

Amount of Inoculated Cells (10^4 /scaffold)	Viability (%-viable cell no. at initial stage)	Albumin Secreted (pg/cell)
14	37.924504	49.690272 ± 4.049649
28	26.150778	35.523805 ± 6.834733
42	25.298302	37.590655 ± 2.815256
56	23.543620	34.181293 ± 0.199821



According to the data of Table 4, the number of viable cells was reduced by about 20-30% after 7 day-incubation in the porous polymeric scaffold and both the viability and the albumin secretion of hepatocytes cultured in the scaffold are lowered as the number of the inoculated cells increases.

As described hereinbefore, the present invention provides a method for preparing biodegradable and biocompatible, porous polymeric scaffolds which are so porous and interconnective among pores as to accommodate and culture cells isolated from the tissues which are to be artificially regenerated in vitro, such as cartilage, bone, liver, heart valve, gastrointestinal duct, urethral canal, etc. The scaffolds serve as excellent matrixes for the artificial culture of various cultures.

In addition, based on the pore formation by the effervescence of salts in the gels prepared from biodegradable polyester polymer and effervescent salt mixtures, the method has an advantage of easily controlling the pore size and porosity of the three-dimensional porous, polymeric scaffolds by controlling the amount and size of the effervescent salts and the concentration of the acidic aqueous solutions by which the effervescence and leaching-off of the salts are induced.

The present invention has been described in an illustrative manner, and it is to be understood that the terminology used is intended to be in the nature of description rather than of limitation. Many modifications and variations of the present invention are possible in light of the above teachings. Therefore, it is to



be understood that within the scope of the appended claims, the invention may be practiced otherwise than as specifically described.



What is claimed is:

1. A method for preparing a biodegradable and biocompatible, porous, polymeric scaffold for tissue engineering, which comprises the steps of:

(i) dissolving a polymer in an organic solvent to prepare a polymeric solution;

(ii) mixing an effervescent salt in the resulting polymeric solution to give a polymer/salt/organic solvent mixed gel;

(iii) removing the organic solvent from the polymer/salt/organic solvent mixed gel; and

(iv) immersing the gel in acidic solution to render the salt to effervesce to yield a polymeric scaffold.

2. A method for preparing a biodegradable and biocompatible, porous, polymeric scaffold for tissue engineering, which comprises the steps of:

(i) dissolving a polymer in an organic solvent to prepare a polymeric solution;

(ii) adding a nonsolvent to prepare the resulting polymeric solution such that the polymer is precipitated and concentrated to form a polymeric gel;

(iii) mixing an effervescent salt in the polymeric gel to give a polymer/salt/organic solvent mixed gel;

(iv) removing the organic solvent from the polymer/salt/organic solvent mixed gel; and

(v) immersing the gel in acidic solution to render to the salt to effervesce to yield a polymeric scaffold.

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3. The method according to claims 1 or 2, further comprising the step of washing the polymeric scaffold following the immersing step.
4. The method according to claim 3, further comprising the step of drying the washed polymeric scaffold following the washing step.
5. The method according to claims 1 or 2, wherein the polymer is a polyester-based polymer.
6. The method according to claim 5, wherein the polyester-based polymer is a aliphatic polyester-based polymer.
7. The method according to claim 6, wherein the aliphatic polyester is selected from the group consisting of poly(L-lactic acid), poly(D,L-lactic acid), poly(glycolic acid), poly(D,L-lactic-co-glycolic acid), poly(caprolactone), poly(hydroxy butyrate), poly(dioxanone) and their copolymers.
8. The method according to claims 1 or 2, wherein the organic solvent for dissolving the polymer is selected from the group consisting of methylene chloride, chloroform, acetone, dimethylsulfoxide, dimethylformamide, N-methylpyrrolidone, dioxane, tetrahydrofuran, ethylacetate, methylethylketone and acetonitrile.
9. The method according to claim 2, wherein the nonsolvent substantially undissolves the polymer.

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10. The method according to claim 9, wherein the nonsolvent is selected from the group consisting of water, ethanol, methanol, aqueous ethanol, isopropyl alcohol, diethyl ether, hexane, heptane and petroleum ether.
11. The method according to claims 1 or 2, wherein the effervescent salt is selected from the group consisting of ammonium carbonate, ammonium bicarbonate, sodium carbonate, and sodium bicarbonate.
12. The method according to claims 1 or 2, wherein the acidic solution is a solution of one selected from the group consisting of citric acid, hydrochloric acid, acetic acid, formic acid, tartaric acid, salicylic acid, benzoic acid and glutamic acid.
13. The method according to claims 1 or 2, wherein the polymer has molecular weight ranging from 5,000 to 500,000.
14. The method according to claims 1 or 2, wherein the effervescent salt has particle size ranging from 100 to 500 μm .
15. The method according to claims 1 or 2, wherein the effervescent salt is mixed at such an amount that the weight ratio of the salt to the polymer ranges from 1:1 to 1:100.
16. The method according to claims 1 or 2, wherein the acidic solution has a concentration ranging from 1% to supersaturated concentration.

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17. A porous polymeric scaffold for tissue engineering characterized in that a porosity and a pore size of the scaffold are varied depending on a concentration of acidic solution, and a particle size and an amount of effervescent salt used in process for preparing the scaffold.

18. The scaffold according to claim 17, wherein the scaffold is prepared in accordance with a method claimed in 1.

19. The scaffold according to claim 17, wherein the scaffold is prepared in accordance with a method claimed in 2.

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