Design of Electrospun Poly vinyl alcohol/Chitosan Scaffoldand Its Cellular Study

Esmaeil Biazar[1,*], Davood Zaeifi[2], Saeed Heidari Keshel [3] , Sanaz Ojani[1], Arefeh Hajiaghaee[1], Roya Safarpour[1], Mahkameh Sheikholeslami[1], Banafsheh Heidari [1], Saba Sadeghpour[1]

[1]Department of Biomaterials Engineering, Tonekabon Branch, Islamic Azad University, Tonekabon, Iran.
[2] Proteomics Research center, Shahid Beheshti University of medical sciences, Tehran, Iran.
[3]Eye research Center, Farabi Eye hospital, Tehran University of Medical Sciences, Tehran, Iran

*Corresponding Author: email address: kia_esm@yahoo.com (E. Biazar)

ABSTRACT

Poly vinyl alcohol/Chitosan nanofibrous mat were prepared by electrospinning method with suitable pore sizes as potential matrices for soft tissue engineering. The designed scaffolds by electrospinning method evaluated by differentanalyses such morphological, mechanical, and cellular analysis.Microscopic results showed diameters of poly vinyl alcohol/Chitosan nanofibers were approximately 150 nm. Mechanical investigations illustratedstress - strain curve of poly vinyl alcohol /chitosan mat indicate good flexibility with average strain and good percentage of yield stress. The cellular resultsrevealthat addition of chitosan to poly vinyl alcohol enhances viability and proliferationof fibroblast cells, which increases the biocompatibility of the scaffold. In fact, addition of a smallpercentage of chitosan to the poly vinyl alcoholproved to be a promising approach for designof a scaffold.

Keywords: Chitosan, Poly vinyl alcohol; Nanofibers; Morphological and Mechanical analyses; Cell culture.

INTRODUCTION

Generally speaking, the chemical composition and structure of tissue engineeringscaffoldsshould be optimized for supporting the reparative process in a particular tissueand for attachment and proliferation of particular cells [1-5]. Fiber-based porous scaffolds, which structurally mimic the extracellular matrix(ECM), have been synthesized from numerous natural or synthetic biopolymers. Thesescaffolds have been specifically engineered by electrospinning platform technology, andwere successfully used for nerve tissue engineering applications [6-9].Electrospinning is a unique technology which canproduce non-woven fibrous structures with fiber diametersranging from nanometers to microns. This range of fiber sizeis difficult to achieve by other fabrication methods [10,11]. Thescaffolds fabricated by these nanofibers possess an extremelyhigh ratio of surface to volume, have adjustable porosity, andcould easily be customized over a wide range of sizes, shapes,and mechanical properties, which makes them very suitablecandidates for tissue engineering [12-17]. Fine-tuning of nanostructuredtopographical cues such as grooves, ridges, pores, and nodesis also important as they influence cell adhesion, migration,proliferation, and differentiation [5].So far, scaffolds have been synthesized from severalnatural and synthetic materials using different fabricationtechniques, including electrospinning. The electrospun biodegradablepolymers were successfully tested for their efficacy tostimulate fibroblast regeneration,taking into account their different structural propertiessuch as the diameter and alignment of the nanofibers [4-23]. Of these, PVA is a non-toxic,hydrophilic, and biocompatible material which has also beenused for other tissue engineering applications [24,25]. Chitosan hasbeen widely used in this field as well [26,27]. However, most ofthe previous studies focus on a single polymer for fabricationof electrospunnanofibrous scaffolds.The high biocompatibility of chitosan has led severalgroups to use different techniques for scaffold

fabrication,such as different three-dimensional shapes including tubularconduits, using chitosan as the base material [28-30]. However,the mechanical properties of these scaffolds are still notoptimal for application in human dermal tissue. It has been shownthat chitosan has quite positive effects on tissuesregeneration [31-33]. Hereconventional electrospinning method was employed to synthesize poly vinyl alcohol /Chitosan nanocomposite fibers and tried to modify the mechanicaland biological properties of poly vinyl alcohol polymer by blendingit with chitosan, and used electrospinning for fabrication ofa biocompatible scaffold.

MATERIALS AND METHODS

The precursor sol for poly vinyl alcohol and chitosan was prepared from polyvinyl alcohol(PVA) (96% hydrolyzed typical average M_w 85,000–124,000 SIGMA ALDRICH) and chitosan (from shrimp shells ≥ 75% (deacetylated) SIGMA ALDRICH). Poly vinyl alcohol /chitosan solution was dissolved in acetic acid. The weight ratio of poly vinyl alcohol /chitosan was fixed at 80:20 in 2%acetic acid. The concentration of the poly vinyl alcohol / chitosan precursor was 5 wt% and mixed under magnetic stirring for 1.5 h at 75 °C. Poly vinyl alcohol / chitosan nanofibrous mat were prepared by electrospinning method. The applied voltage was 21-25 kV and the elecrospinning distance was fixed at 10 cm. The characteristic morphology and diameter of Poly vinyl alcohol / chitosan nanofibrous mat were characterized by scanning electron microscopy (SEM).Tensile strength of nanofibrousmats were tested using INSTRON (5566, USA) instruments series II Automated materials testing system. The mats were cut into dumbbell shaped strips with 40 mm length and 5mm inner width. The thicknesses of the mats were measured using the electronic digital micrometer screw gauge. The sample strips were held by pneumatic grips and the tensile force were applied at an extension rate of $2mm.min^{-1}$. The tests were conducted at 20 ±2°C in 65±5% relative humidity. Triplicates were tested for each type of electrospunnanofibrousmat.

Cellular analysis

For cytotoxicity analysis, fibroblast cell suspension (L929) from mouse tails was prepared according to International Organization for Standardization 10993 standards. The control sample (TCPS; Tissue Culture Poly Styrene) were well cleaned and sterilized by the autoclave method. Individual samples were placed in Petri dishes using a sterilized pincer; 3 cc of the cell suspension wasremoved by pipette and poured into the control and experimental samples. Thereafter, all of the samples were placed separately in a Memmert incubator at 37°C for 24 and 48 hours. Cell proliferation was determined by the MTT assay for viable cell numbers. The MTT tetrazolium compound was reduced by living cells in a colored formazan product that was soluble in the tissue culture medium. The quantity of formazan product was directly proportional to the number of viable cells in the culture. The assays were performed by adding 1 mL of MTT solution (Sigma-Aldrich) and 9 mL of fresh medium to each well after aspirating the spent medium and incubating at 37˚C for 4 hours with protection from light. Absorbance was measured at 570 nmusing a Rayto micro-plate reader.

Five samples were performed for each biomaterial.For microscopic study, the cultured mats with cells were washed by PBS and then fixed by glutheraldehyde (2.5%) at 4 ° C for 2 h. The samples were dehydrated by alcohols and then kept with tetraoxide osmium vapors at 4 ° C for 2 h.The samples were coated with gold and investigated by a scanning electron microscope (TScan,VEGA,Czech).All data are expressed as mean standard error of the mean unless noted. One-way analysis of variance with post hoc Tukeymeans comparison tests and unpaired Student's t-tests were conducted with a significance level of $p<0.05$. A minimum of three replicate samples were used for all experiments.

RESULTS

Figure 1 shows SEM images ofpoly vinyl alcohol, chitosan,andpoly vinyl alcohol /chitosan nanofibers prepared from the precursor.

The composite systems consist of nanofiber and micro-beadstructure. All images reveal that designednanofibers have good uniformity

withregular diameter of about 100, 250 and 150 nm for poly vinyl alcohol , chitosan, and poly vinyl alcohol /chitosan respectively.

Mechanical properties

Figure 2a shows the stress - strain curve of poly vinyl alcohol nanofibrous mat which reveals that the sample was flexible. The percentage of strain was high however the yield stress was low.The tensile strength was 1.2 MPa and the amount of displacement was 27%.The sample was measured on the basis of the value of the modulus of elasticity. Figure 2b shows the stress - strain curve of chitosan nanofibers which reveal the low flexibility but high strain percentage, the tensile strength was 22 MPa and amount of 18% displacement. Chitosannanofibrous mat wasmeasured on the basis of the value of the modulus of elasticity too. Figure 2c represent the stress - strain curve of poly vinyl alcohol /chitosan mat indicate good flexibility with average strain and good percentage of yield stress, the tensile strength were 11 MPa and the amount of displacement was 21%.

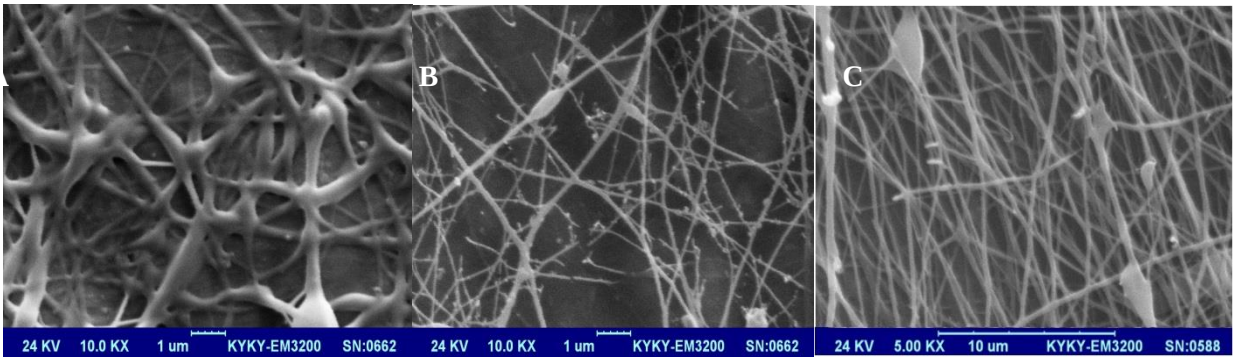

Figure 1. SEM images of poly vinyl alcohol (A), chitosan (B) and poly vinyl alcohol / chitosan nanofibers (C).

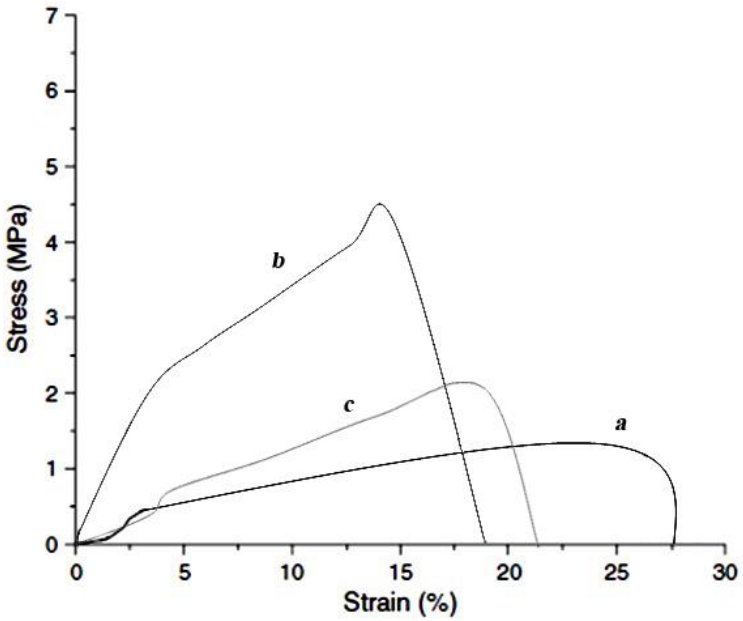

Figure 2. Tensile stress-strain curve of nanofibrousmats.a)poly vinyl alcohol , b)chitosan , and c)poly vinyl alcohol / chitosan.

Cellular results

In this study, MTT assay was performed to evaluate thecell proliferation rate of the fibroblastcells on the poly vinyl alcohol, chitosan andpoly vinyl alcohol /chitosan nanofibrousmats. As shown in figure 3,the proliferation of cells on poly vinyl alcohol /chitosan mats washigher than on poly vinyl alcohol mats, indicating that the chitosanmight have accelerated the proliferation rate of the cells. This could be attributed to the higher surface amineand the lower water contents of the swollen poly vinyl alcohol /chitosan mat. In addition, it has been shown that fiber diametercan influence cell adhesion, proliferation, and migration (Figure 4).It was shown by Bashur et al thatsmaller fiber diameters favor better fibroblast cell attachmentand proliferation of cells cultured on non-woven electrospun fiber meshes [36].

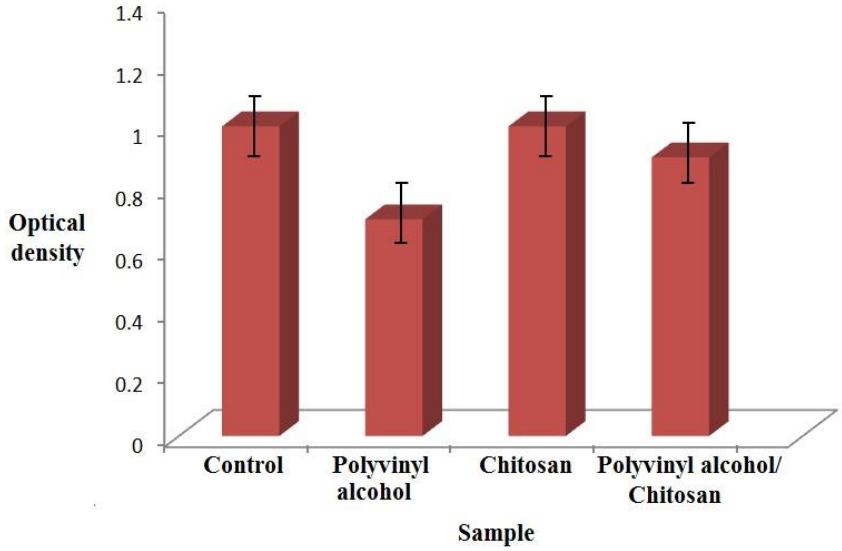

Figure 3. MTT analysis of fibroblast cell culture on nanofibrousmats. Poly vinyl alcohol, chitosan, poly vinyl alcohol / chitosan nanofibrous mats, and control (TCPS).

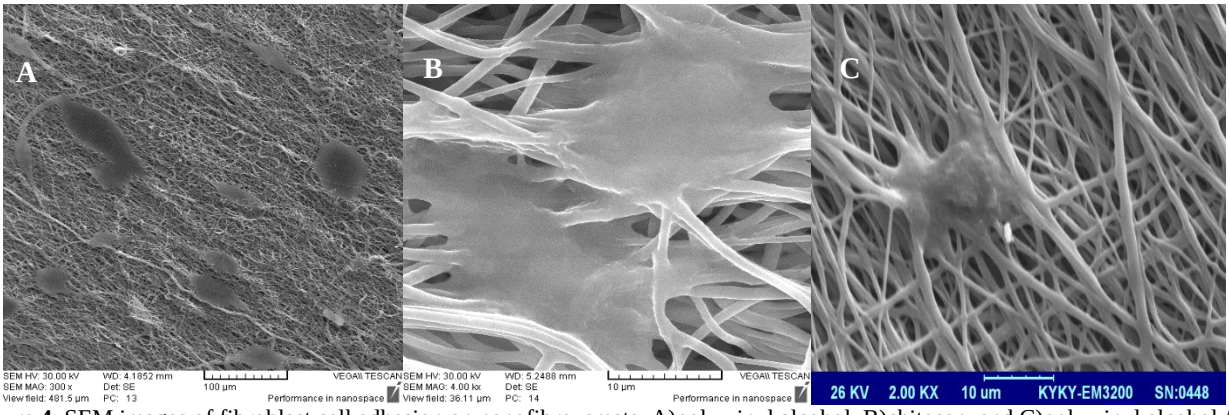

Figure 4. SEM images of fibroblast cell adhesion on nanofibrousmats. A)poly vinyl alcohol, B)chitosan, and C)poly vinyl alcohol / chitosan.

Some natural materials such as collagen, chitosan,alginate, some carbohydrates and peptides have beenreported as scaffold modifiers. Chitosan has been provedand regarded as biodegradable noncytotoxic material, whichhas some interesting biological activities [8,13].The high biocompatibility of chitosan has led severalgroups to use different techniques for scaffold fabrication,such as different three-dimensional shapes including tubularconduits, using chitosan as the base material. However,the mechanical properties of these scaffolds are still

notoptimal for application in tissue regeneration[31-33].

Here, we tried to modify the physicochemicaland biological properties, especially mechanical property ofchitosan polymer by blendingit withpoly vinyl alcohol, and used electrospinning for fabrication ofa suitable scaffold.In our study, poly vinyl alcohol/Chitosan fibers by conventional electrospinning method designed and tried to modify the mec hanical and biological properties of poly vinyl alcohol polymer by blendingit with chitosan.Chitosan nanofibrous mat measured on the basis of the value of the modulus of elasticity too. Results demonstrated the poly vinyl alcohol /chitosan nanofibrousmat indicate good flexibility with average strain and good percentage of yield stress.Interestingly, it was shown that the porosity of the samelayers in the mats fabricated with poly vinyl alcohol and poly vinyl alcohol /chitosan blend did not differ significantly.

However, the pore morphologieswere different. As mentioned above, poly vinyl alcohol solutionyielded fibers with relatively large diameters, which formedpores larger than those formed by small-diameter poly vinyl alcohol /chitosan fibers.Poly vinyl alcohol /chitosan mats had a higher number of pores.It should be noted that porosity and pore morphologyare important for many tissue engineering applications asthey allow migration of the cells and growth of blood vesselsacross the scaffold and ensure effective exchange of nutrientsand waste products between the cells and their microenvironment.

CONCLUSION

In this study, poly vinyl alcohol, chitosan and poly vinyl alcohol /chitosan nanofibrous mats were prepared by electrospinning method.

The poly vinyl alcohol /chitosan mats havebeen found to exhibit better mechanical propertiescompared with poly vinyl alcohol or chitosan mats.

Cellular investigations showedbetter adhesion, growth, and viability in the chitosan content nanofibers than in the poly vinyl alcohol nanofibers. Thesepoly vinyl alcohol / chitosan mats could be used well for soft tissueengineering.

REFERENCES

1. Samadikuchaksaraei A. Scientific and industrial status of tissue engineering.AfrBiotechnol 2007;6(25):2897-2909.

2. Chew SY,Mi R,Hoke A, Leong KW. The effect of the alignment of electrospun fibrous scaffolds on Schwann cell maturation. Biomaterials 2008;29(6):653-661.

3. Bhattarai N, Edmondson D,Veiseh O,Matsen FA, Zhang M. Electrospun chitosan-based nanofibers and their cellular compatibility. Biomaterials 2005;26(31):6176-6184.

4. Majdi A,Biazar E,Heidari S. Fabrication and comparison of electro-spun PHBV nanofiber and normal film and its cellular study. Orient. J. Chem 2011;27: 523-528.

5. Biazar E. Polyhydroxyalkanoates as potential biomaterials for neural tissue regeneration. Int. J. Polym Mater and PolymBiomater 2014;63: 898-908.

6. Rezaei-Tavirani M,Biazar E, AI J,Heidari S,Asefnejad A. Fabrication of collagen-coated Poly (beta-hydroxy butyrate-cobeta-hydroxyvalerate) nanofiber by chemical; and physical methods. Orient. J. Chem 2011;27: 385-395.

7. Ai J,Heidari SK,Ghorbani F,Ejazi F,Biazar E,Asefnejad A, et al. Fabrication of coated-collagen electrospun PHBV nanofiber film by plasma method; and its cellular study. J. Nanomater 2011;2011: 1-8.

8. Biazar E,Heidari SK. Chitosan–Cross-linked nanofibrous PHBV nerve guide for rat for sciatic nerve regeneration across a defect bridge. ASAIO J 2013;59:651-659.

9. Sahebalzamani M,Biazar E,Shahrezaei M,Hosseinkazemi H,Rahiminavaie H. Surface modification of PHBV nanofibrous mat by laminin protein and its cellular study. Int. J. Polym Mater and PolymBiomater 2015;64(3):149-154.

10. Biazar E. Use of umbilical cord and cord blood-derived stem cells for tissue repair and regeneration. Expert Opin. Biol. Ther2013;13(12):1653-1662.

11. Biazar E,Sahebalzamani A. Modification of Poly CaprolactoneNanofibrous Mat by Laminin Protein and Its Cellular Study.J BiomaterTissEng 2014;4:423-429.

12. Hosseinkazemi H,Biazar E,Bonakdar S,Ebadi MT,Shokrgozar MA,Rabiee M. Modification of PCL ElectrospunNanofibrous Mat With Calendula officinalis Extract for Improved Interaction With Cells. Int. J. Polym Mater and PolymBiomater 2015;64:459-464.
13. Biazar E,Heidari S. Effects of chitosan cross linked nanofibrous PHBV scaffold combined with mesenchymal stem cells on healing of full-thickness skin defects. J.Biomed.Nanotechnol 2013;9(9):1471-1482.
14. Biazar E,Heidari S. Gelatin-modified nanofibrous PHBV tube as artificial nerve graft for rat sciatic nerve regeneration. Int. J. Polym Mater and PolymBiomater2014;63:330-336.
15. Biazar E,Heidari S. Design of an oriented porous polymeric guide for neural regeneration. Int. J. Polym Mater and PolymBiomater 2014;63: 753-757.
16. Biazar E,Heidari S. Unrestricted Somatic Stem Cells Loaded in Nanofibrous Scaffolds as Potential candidate for Skin Regeneration. Int. J. Polym Mater and PolymBiomater2014;63:741-752.
17. Biazar E,Heidari S. Behavioral evaluation of regenerated rat sciatic nerve by a nanofibrous PHBV conduit filled with Schwann cell as artificial nerve graft. Cell CommunAdhes 2013;20(5):93-103.
18. Biazar E,Heidari S. Rat sciatic nerve regeneration across a 30-mm defect bridged by a nanofibrous PHBV and Schwann cell as artificial nerve graft. Cell CommunAdhes2013; 20(1-2):41-49.
19. Biazar E,Heidari S. Rat Sciatic Nerve Reconstruction Across a 30 mm Defect Bridged by an Oriented Porous PHBV Tube With Schwann Cell as Artificial Nerve Graft. ASAIO J 2014; 60:224-233.
20. Biazar E,Heidari SK,Pouya M, Rad H,Omraninava M,Azarbakhsh M , et al.Nanofibrous nerve conduits for repair of 30-mm-long sciatic nerve defects. Neural Regen Res 2013;8(24):2266-2274.
21. Biazar E,Heidari SK,Pouya M. Efficacy of nanofibrous conduits in repair of long-segment sciatic nerve defects. Neural Regen Res 2013;8(27):2501-2509.
22. Bini TB,Gao S, Wang S, Ramakrishna S. Poly(l-lactide-co-glycolide) biodegradable microfibers and electrospunnanofibers for nerve tissue engineering: an in vitro study. J Mater Sci. 2006;41(19):6453-6459.
23. Ito S, Wang W, Tsuneo O. Efficacy of chitosan nanofiber mesh as a scaffold for regenerating nerve tissue. KichinKitosanKenkyu 2006;12:194-195.
24. Patel S,Kurpinski K, Quigley R. Bioactive nanofibers: synergistic effects of nanotopography and chemical signaling on cell guidance. Nano Lett 2007;7(7):2122-2128.
25. Venugopal J, Zhang Y, Ramakrishna S. In vitro culture of human dermal fibroblasts on electrospunpolycaprolactone collagen nanofibrous membrane.Artif Organs 2006;30:440-446.
26. Lee CH, Shin HJ, Cho IH, Kang YM, Kim IA, Park KD,et al .Nanofiber alignment and direction of mechanical strain affect the ECM production of human ACL fibroblast. Biomaterials 2005;26:1261-1270.
27. Chen JP, Chang GY, Chen JK. Electrospun collagen/chitosan nanofibrous membrane as wound dressing. Colloids Surf A: PhysicochemEng Asp 2008;313:183-188.
28. Sahoo S,Ouyang H,Goh JCH,TayTE,Toh SL. Characterization of a novel polymeric scaffold for potential application in tendon/ligament tissue engineering. Tissue Eng 2006;12(1):91-99.
29. Zheng-Qiu G,Jiu-Mei X, Xiang-Hong Z. The development of artificial articular cartilage – PVA-hydrogel. Biomed Mater Eng1998;8(2):75-81.
30. Miyashita H,Shimmura S, Kobayashi H. Collagen-immobilized poly (vinyl alcohol) as an artificial cornea scaffold that supports a stratified corneal epithelium. J Biomed Mater Res B ApplBiomater 2006;76(1):56-63.
31. Wang A,Ao Q, Cao W. Fiber-based chitosan tubular scaffolds for soft tissue engineering: fabrication and in vitro evaluation. Tsinghua SciTechnol 2005;10(4):449-453.
32. Izume M,Taira T, Miyata T. A novel cell culture matrix composed of chitosan and collagen complex. In: Skjack–Break G, Authonsen T, Sandford P, editors. Chitin and chitosan. London: Elsevier Applied Science. 1989, P 653-656.
33. Koyano T, Minoura N, Masanobu N, Kobayashi K. Attachment and growth of cultured fibroblast cells on PVA/chitosanblended hydrogels. J Biomed Mater Res 1998;39:486-490.