IJBPAS, July, 2015, 4(7): 303-316

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

STUDY OF CHITOSAN-POLYETHYLENE OXIDE NANOFIBERS AND THEIR CELLULAR COMPATIBILITY BY BONE MARROW STROMAL CELLS

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ABSTRACT

Several studies have been performedto achieve a biodegradable and biocompatible scaffold for growing and proliferation stem cells. The aim of this study was to evaluate the ability of bone marrow stromal cells (BMSCs) for growing, proliferation, viability andno differentiation on a biodegradable scaffold of Chitosan(Ch) / polyethylene oxide (PEO) nanofibrous and Chfilm for using in tissue engineeringandcell therapy.

First, a thin film Chwas prepared by casting method. For preparation nanofibers,Chitosan-PEOcomposite mixture with a ratio of 90 to 10 and 80 to 20 by electro spun and then were studied by scanning electron microscope (SEM). These scaffolds were located on 1% gelatin at24 -well plates and then weresterilized. Femoral BMSCs of rats were cultured on scaffolds after three passages in the empty house plate as control BMSCs were then culturednanofibrous membranesChandthin film chitosan. The rate of proliferation, No differentiation, viability and cell shape BMSCs at the second,fourth and sixth were studied.

The results showed thatthe morphology of cells maintained on the scaffoldswas similar to the control group. Meancell proliferationrate of co-cultured BMSc in the scaffoldson consecutive daysincreasedwhen compared to control group but the differences were not significant. Mean percentage of viability of BMSCsin all nanofiber groups were similar to control group. The

results also showed thatat the endofthesix days mean percentages of differentiation, and apoptosis of BMSC son then anofiber groups were similar to cultured cells in the control group. No significant differences were shown between Ch/PEO nano fibers composition of 90 to 10and 80 to 20.

Study of BMSCs proliferation, morphology, viability, differentiation and morphologyon biodegradable Ch/PEO nano fibers and thin film Chscaffolds are obtaining a model which can be used in tissue engineering and cell therapy.

Key words:Nanofibers, Chitosan, Scaffolds, BMSCs.

INTRODUCTION

The culture of the Cellson a biodegradable and biocompatible scaffold can provide a relatively new approach to replace the damaged tissues. Regenerative medicine and tissue engineering require twocomplementary key ingredients:1) biologically compatible scaffolds that can be readily adopted by the body system without harm, and 2) suitable cells including various stem cells or primary cells that effectively replace the damaged tissues without adverse consequences.The scaffold should mimic the structure and biological function of the native extracellular matrix (ECM). A well known feature of the native ECM structures is the nanoscaled dimensions of their physicalstructure [1]. Design of ananofibrous is of important concern in the effective application of these nanostructured materials[2].Biological achievements regarding cell culture using biodegradablematerials are more promising techniques.A number of manufacturing processes have been explored to fabricate micro or nanoscale fibers matrices, including drawing, self-assembly, template-directed synthesis, phase eparation, and electro spinnin[3]. Among these techniques, the electro spinning has been widely accepted as the simplest and least expensive means to fabricate ultrafine fibers [4].Chitosan, known as biopolymer, is of interest in cartilage research. Previous research has shown that Chis biocompatible and biodegradable [5]and used in drug delivery systems [6], and various tissue engineering applications [7-8]. The Chscaffold for the bone tissue engineering has been widely investigated [9].

In a more recent report, polyethylene oxide PEO was introduced in Chto fabricate ultrathin hybrid nanofibers, which showed that the spin ability of Chsolutions depends strongly on the mass ratio of Chto PEO. It is used to reduce the viscosity of Chsolution so that the solution is able to spin at high

polymer concentrations. PEO is a biocompatible polymer and one of the few syntheticpolymers approved for internal use in food, cosmetics, personal care products and pharmaceutical. To obtain better fiber structures at high Ch/PEO ratios, which is often desirable for tissue engineering applications, Triton X-100™ was introduced into the solution as a nonionic surfactant. Finally, di-methyl-sulphoxide (DMSO) was introduced into the solution as a co solvent to improve processing conditions and increase fiber yields by relaxing Chchain entanglement[10]. To study the cellular compatibility of the nanofibrous structure for potential use in tissue engineering, BMSCs were seeded on the nanofibrous and thin film Chand cultured for up to 6 days.for preparation of thin Chfilm,it is better to dissolve Chin an organic weak acid solution to form a viscous solution. The solution is cast onto a soft surface. Knowing the properties of Chmembranes are required to explain their effect on cell growth and reproduction. Viscosity of solution, physical dimensions of the membrane, Young's modulus, yield strength, fatigue and strain rate can affect the properties of D-acetylated Chmembrane [11].

BMSCs are multipotential stem cells capable of differentiationinto numerous cell types including fibroblasts,bone, cartilage, and muscle cells[12]. BMSCs are not lineage-restricted whentransplanted into foreign tissues they produce functionalno hematopoietic cell types [13–14]. In addition, BMSCs arecapableof producing a variety of cytokines and hematopoieticgrowth factors [15]. Because of their plasticityto differentiate into tissues other than their ownderivation, BMSCs have been used for restorative therapyof stroke, myocardial infarctions, and osteogenesisimperfect [16–17].BMSCs can be isolated from the whole bone marrow as they adhere to plastic culture dishes. Recentstudies have shown that BMSCs can be induced to express a neuronal phenotype in-vitro under specificexperimental conditions[18].It has recently been established that in several tissues, adult stem cells may undergo a fate other than that usually manifested in physiological conditions. As an example, stem cells isolated from bone marrow stromal cells (BMSCs) can differentiate not only into blood cells, but also into hepatocytes,skeletal muscle,and cardiomyocytes [19]. The purpose of the study was to provide a biodegradable scaffold of Ch/PEO with the ability for growing, proliferation and undifferentiation of BMSCs.

2.Experiments

2.1Electrospinning

At first, two percent Ch(Mw=190kD) (Sigma, USA) solution and three percent PEO (Mw=9×10^5g/Mol) (Sigma, USA) solution were prepared separately by dissolving Chor PEO in 0.5 M acetic acid. The Chand PEO solutions of different proportions were then mixed to obtain the mixtures with weight ratios of Ch/PEO

ranging from 80/20and 90/10 and the resultant mixtures were stirred for 24 h. Solutions containing 0–0.5 wt% of Triton X-100™ and 0–10 wt% of DMSOwere mixed with Ch/PEO solutions, and the mixtures were stirred overnight and centrifuged to remove air bubbles before use[10].

The stock solution for electrospinning (Farasan Co, Iran) was fed into a 3 ml disposable syringe fitted with a pipette tip of 0.5 mm in diameter. The solution feed was driven by the gravity and the feed speed was controlled by the tilt angle of the syringe. A DC voltage of 18–20 KVwas applied between the syringe tip and a cylindrical collector covered with aluminum foil. The cylinder had a diameter of 10 cm and was driven by a DC motor with controllable speed. The typical distance between the syringe tip and the grounded collector was 12–15 cm. During the spinning process, the pendant droplet at the syringe tip was split by a repulsion force set by the charge in the droplet, and formed a jet of a cone-like shape traveling towards the collector, during which the solvent evaporated and polymer fibers deposited on the collector in the form of a non-wovenfiber mat. All the spinning experiments were performed at room temperature. The as-spun nanofibrous were dried under vacuum at room temperature. Electro spunnanofibrous were sputter-coated with Au/Pd, and the morphology of the nanofibrous was examined with an SEM(JEOL JSM-840A) at an accelerating voltage of 10 KV. The average diameter of electro spunnanofibrous was determined by measuring the diameters of the nanofibrous at 20 different points in a 645×484 SEM image. The diameters were presented as the average ± standard deviation.

2.2.Technique of thin Ch membranepreparation

In preparation of eachmembrane using the method described by Cheng [20]. Briefly, first, the Chlow molecular weight (448869, DDA= 75% -85%)(Sigma, USA) with a ratio of 1% weight was added to water twice at $40^{\circ C}$. The rate of 0.5ml of acetic acid was added and heated for 2 hours on the machine - the magnetic stirrer was placed perfectly stirred up. Then bubbles inside the solution centrifuged at 2500rpm for 10minutesand was placed the second. Then 7.5 ml was poured into a Petri dish with a diameter of 75 mm

and was placed for 24hours at 25℃ to evaporate the solvent, the membrane is formed. Then washed with distilled water twice and dried at room temperature for 25hours.

2.3.Preparations of BMSCs

The Animal Studies Ethical Committee at BaqiyatallahUniversity approved the experimental work done in this study. BMSCs were collected from the tibias and the femurs of adult Sprague–Dawley rats at 6–8 weeks of age (Neuroscience Research Center, Tehran, Iran). The proximal and distal ends of the bones were removed under aseptic conditions and the bone marrow was aspirated with 5 ml of α-MEM(Gibco, UK) containing 500 units of heparin using a 21G needle. The cell pellet was obtained and suspended in α-MEM (containing 15% fetal bovine serum (FBS), 100 U/ml penicillin,100 µg/ml streptomycinand 25 ng/ml amphotericin B, and 2 mM L-glutamine: all from Gibco, UK). The harvested cells were seeded on a 75 cm^2 flask at 37℃, 5% CO2 incubator for 24 hours. The flasks were washed with PBS in order to remove the hematopoietic cells. The cells were incubated for 2–3 days, where the cells reached the confluence and the culture was repeated for three passages (P3), one week for each passage. The cells were then removed with 0.25% trypsinand 1 mM EDTA for 5–10 minutes at37℃in order to obtain a single-cell suspension.The method obtains ofBMSCs used in this study similar to that reported previously [21].

2.4.Cell culture and adhesion

Nanofibrous membranes and thin film Chwere deposited on 24-well culture plates and washed several times with 75% ethanol for sterilization and washed with deionized water and PBS at neutral pH to remove residual solvent and surfactants introduced during the electrospinning. 10^5 of BMSCs cell culture medium containing10% FBS, 100 IU ml^{-1} penicillin, and 100 µg ml^{-1} streptomycin were then seeded onto nanofibrous membranes and thin film Chin 24-well culture plates.

2.5.Cell Proliferation

To evaluate the growth and proliferation of BMSCs, at first, all photoswere takenwith different magnifications of each of the houses on the second, fourth and sixth days by digital camera attached to aninverted microscope. To count the cells, five microscopic fields with objective lens 10Xwere randomly selected and the number of cells per field were counted and recorded.

2.6.Cell viability

At the end of the sixth day, to determine the cell death, 100 ml of Acridin Orange (2mg/ml) (Sigma, USA) was added to each of

the houses under the fluorescence microscope, one hundred cells were counted and photographs were then taken with a digital camera. Orange-red nuclei and redcytoplasm of cells were as dead cells and cells with green nucleus and cytoplasm were considered as viable cells.

2.7.Immunocytochemistrical study

BMSCs have the potential to differentiate into various cells and can transdifferentiate into other cells, it means no differentiation of BMSCs were occurring. For this purpose, fibronectin, CD44 and CD45antibodies were used as described below:The BMSCs were washed in PBS, and fixed with 4% paraformaldehyde (Invitrogen, UK) in PBS for 15 minutes. The fixed cellswere washed twice with PBS before staining. Permeabilizationand blocking nonspecific antigen reaction werecarried out in blocking buffer consisting of 0.1% TritonX-100 and 10% goat serum in PBS for 1 hour.The primary antibodies: mouse anti-fibronectinmonoclonal antibody (1:100; Chemicon, USA), mouseanti-CD44 monoclonal antibody (1:300; Santa Cruz Biotechnology, USA), mouse anti-CD45 monoclonal antibody (1:300; Santa Cruz Biotechnology, USA) were incubated overnightat $4^{\circ C}$ washed three times in PBS, incubated withthe relevant secondary antibody (anti-mouse DAB conjugatedfrom Chemicon, USA) for 2 hours at room temperature,washed in PBS twice. Then, they were washed in PBSand examined using a microscope at 200X and 100Xmagnifications (Axiophot, Zeiss, Germany). For negativecontrols, the primary antibodies were omitted andthe same staining procedure was conducted as above. The method Immunocytochemi used in this study similar to that reported previously [21].

2.8.Cell Morphology

At the end of the sixth day, morphology of BMSCs were studied using hematoxylin-eosin stainingmethod. The slides were then examinedby alight microscope.

2.9.Statistical Analysis

The number of independent replicas is listed individually for each experiment. Where applicable, all data are expressed as mean ±standard deviation. Student's t-test and single factor ANOVA were used for parameter estimation and hypothesis testing with $P<0.05$ considered as being statistically significant.

3.RESULTS

3.1.Electrospinning

The solution viscosity is a critical factor that affects on solution spin ability and morphology of as-spun fibers [4 and 10].Figure 1 shows the SEM image of the

electro spun structure when 0.3 wt% of Triton X-100™ andDMSO was introduced in the solution at a polymer concentration 2wt% of Chwithout PEO Compared to the Ch /PEO ratio of 90/10 and 80/20. The addition PEO improved the electro spun structure and Chwithout PEO fibers structure was produced the bead-like structure was embedded in the fibers.The average fiber diameters, as estimated from the images, were 110±24 nm and 115±29 nm, respectively.

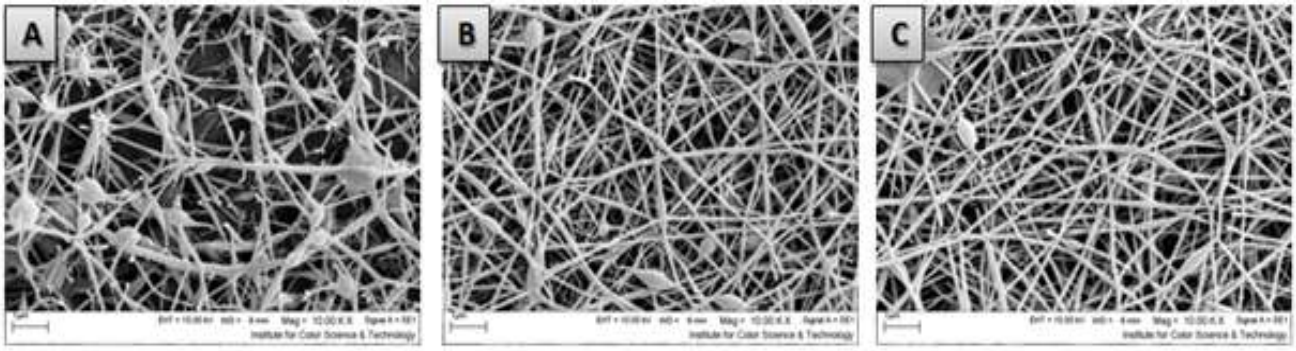


Fig 1.: SEM images of electro spunstructures prepared from solutions containing: 0.3% Triton X-100™ and 10% DMSO. (A) 2% and 100/0; (B) 2.05% and 90/10; (C) 2.1% and 80/20.

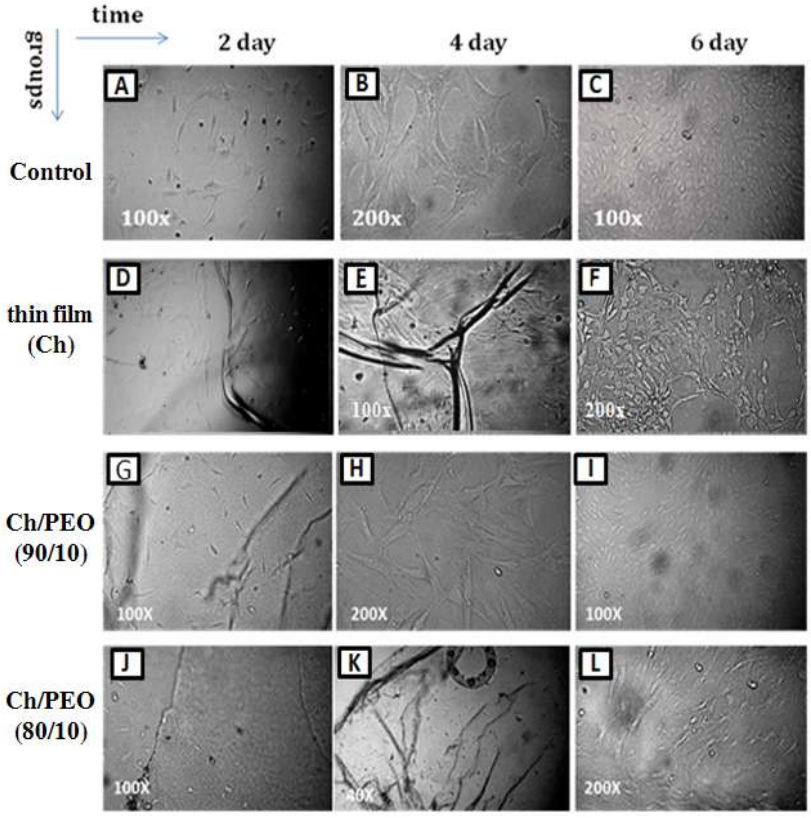
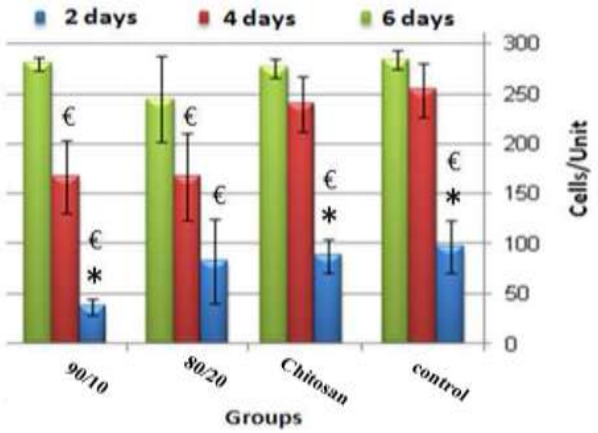


Fig 2. The rate of vivid cells by inverted microscope on the second, fourth and sixth day on the thin film Ch, nanofiber 90/10 and nanofiber 90/10.



*** significantly differences with fourth day**
€ significantly differences with sixth day

Table 1: Comparison of the percentage of cell proliferation on control, the thin film Ch/PEO (90/10) and Ch/PEO (80/20) groups on different days. The percentage of cell proliferation on the second day on the control, the thin film Ch/PEO (90/10) was significant differences on the fourth and sixth days, although was not significant differences between the second day with the fourth day and was significant differences the second day and fourth day with the sixth day 80/20 of nanofiber group. No significant differences were found on the fourth and sixth days to control and thin film Ch groups, although in the Ch/PEO (90/10) group was significant differences between the fourth and sixth day.

3.2. Cell Proliferation

The Percentage of proliferation in the control group on the second day was 40%, on the fourth day was 85% and on the sixth day was near 100%.

The Percentage of proliferation on the thin film Ch on the second day was 35%, on the fourth day was 80% and on the sixth day was 95%.

The percentage of multiplied cells on nanofiber 90/10 on the second day was 15%,on the fourth day was 55% and on the sixth day the entire surface plate was full and whole cells were sticking together and pressed.

The rate of growth and proliferation of cells on nanofiber 80/10 on the second day was 25%,on the fourth day was 55% and on the sixth day more than 95% of plate was full,although significant differences were not seen between the two groups. (Figure 2 and Table 1)

3.3.Cell Viability

This was accomplished by culturing BMSCs cells on nanofibrous membranes in cell culture medium. Figure3 shows fluorescence images of BMSCs cells cultured on a fiber membrane as well as on a Chfilm of the same Ch/PEO (90/10) and Ch/PEO (80/20). Live cells, stained green, appeared to adhere well and exhibited a normal morphology on both surfaces. Dead cells, stained red or orange, were found on both surfaces.Nosignificant differences were observed in cell viability among the groups after six days with small percentage of cell apoptosis**.**

4.3.Cell Differentiation

The mean percentages of immunoreactive cells to fibronectin and CD44 at the P3 were 92.75 ± 3.86%, 94.3 ± 4.66 (mean ± SE) respectively. None or very few cells were immunoreactive for CD45 (hematopoietic marker). The immune reactivity of the undifferentiated BMSCs to fibronectin and CD44 antibodies in P3 is provided in Figure 4.

5.3.Cell morphology

In order to assess the morphology and cytoskeletalarchitecture of BMSCs cells on the different substrates. The cells were permeabilized and stained with hematoxilin and eosin for nuclei and cytoskeleton, respectively, and then visualized using a light microscope as detailed in Materials and methods.

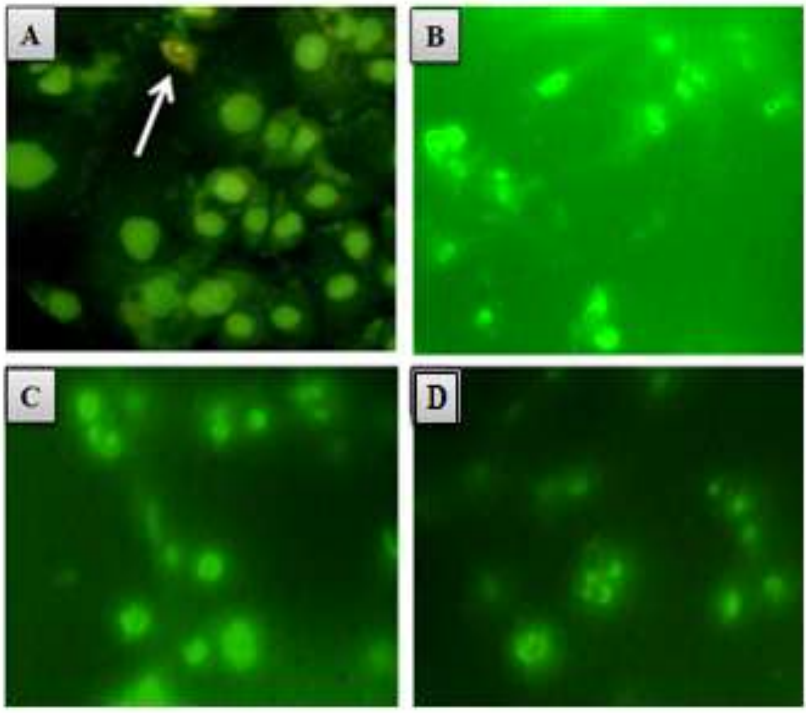


Fig 3: Staining of live (green) and dead (orange) of BMSCs cultured for 6days on (A) control (B) Chfilm (C) nanofibrous membrane of Ch/PEO (90/10) and (D) nanofibrous membrane of Ch/PEO (80/20).

Table 2. Mean percentageof viability after 6 days

Groups	Mean percentageof viability for 6 day
Control	3.2±0.2
Thin film Ch	2.4±0.5
Ch/PEO (90/10)	3.2±0.6
Ch/PEO (80/20)	2.4±0.3

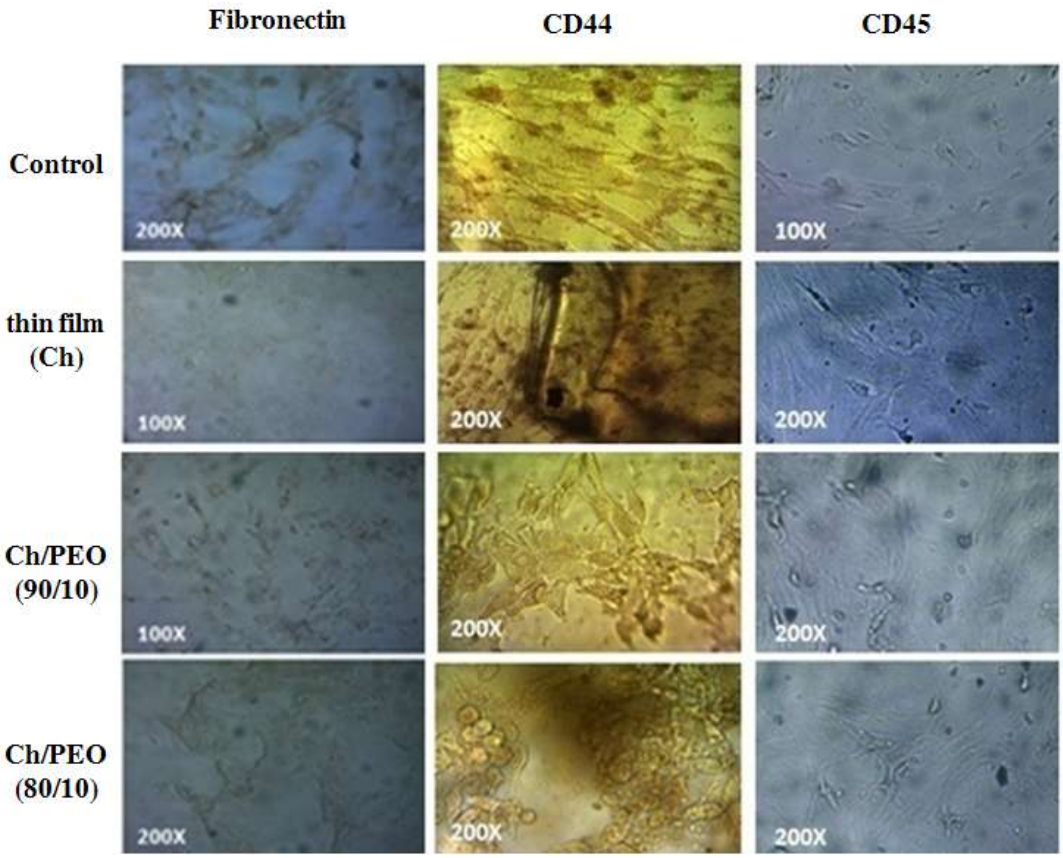


Fig 4. Characterization of the undifferentiated bone marrow stromal cells usingimmunocytochemistry. The differentiation markers used in the study included fibronectin(a marker for BMSCs) and CD44 (markers of mesenchymal stem cells). Theywere incubated with anti-fibronectin, anti-CD44 (primary antibodies),followed by the secondary stained with DAB reagent.

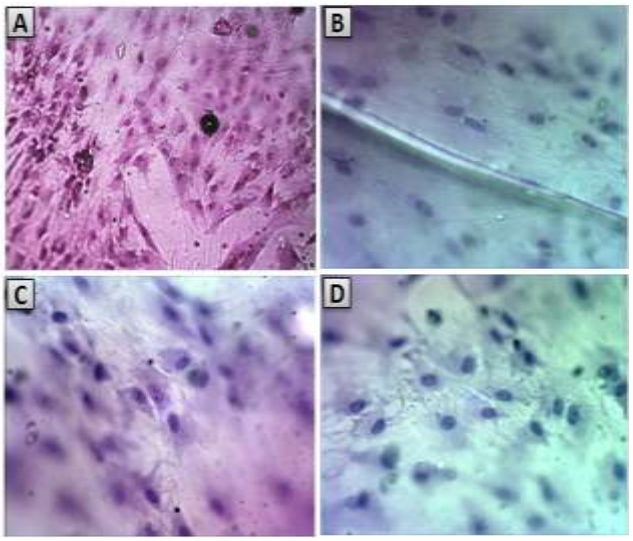


Fig 5. The cells stained with Hematoxilin and Eosin in the control (A), Ch (B), Ch/PEO (90/10) (C) and Ch/PEO (80/20) (D).

4.CONCLUSION

The characteristics of intermolecular interactions between polymer chains is called the viscosity of a polymer solution.

The strong hydrogen bonding between NH_2 and OH groups of Ch polymer chains is the reason of high viscosity of Ch solution.

By adding PEO, the viscosity decreases which can be attributed to the change in inter and intermolecular interactions of Ch chain.

PEO molecules bound onto Ch backbone disrupt the self-association of Ch chains as a result of forming new hydrogen bonding between its OH groups and water molecules.

Physically, this modulation in associative forces by PEO proves an increase in Ch solubility and a decrease in its solution viscosity. PEO has a high solubility in water, and electro spun pure PEO fibers membranes dissolve quickly in water at $37^{\circ C}$.

Thus, it is of practical interest to study the effect of the amount of PEO in Ch /PEO nanofibers on the integrity of the fibers structure in water. Similar approaches have been reported in using polar or non polar solvents to improve the electro spin ability of polymer solutions [4], [10] and [22].

Interactions between cells and engineered ECM are crucial for modulating or redirecting cell functions in an invitro environment. Biocompatibility of tissue-engineered scaffolds is of primary concern since it affects cell attachment, proliferation, and further growth [23].

Dezawa et al reported that BMSCs satisfied the above criteria [24]. In addition, BMSCs are easily accessible, readily adhere to plastic culture dishes [25], also by appropriate induction, they can differentiate into other lineages which include glial cells [26]; so, BMSCs can be a feasible source for cell replacement therapies [27].

In this study, to determine the purity of stromal cells, fibronectin glycoprotein were shown in mesenchymal cells; then, BMSCs were stained using immunocytochemistry method.

High expression of this protein was detected in these cells. To confirm the purity of BMSCs cells, this method has been used by other researchers in the CD44 antibody [28].

Other researchers have also shown the results in human mesenchymal stem cells [29].

Lamoury et al have also cultured animal and human BMSCs in two separate environments using anti-fibronectin antibody and gene expression of Oct-4 mRNA and confirmed the mesenchymal origin of these cells [30].

In a different study the proliferation and differentiation potential of human bone marrow mesenchymal stem cells into hepatocytes on nano-scaffolds composed of

poly Poly (ε-caprolactone) (PCL), collagen and polyethersulfone (PES) was shown.[2].

According to cell line, the human cell type was used in their study is similar to cells have been used in our investigation but their main purpose was to differentiate these cells into hepatocytes.

In conclusion, it seems BMSCs were grown without difficulty and proliferate on Ch/PEO nanofiber. on the other side, few cell death of BMSCs were shown when they co-cultured with Ch/PEO nanofiber. Viability, morphologyand undifferentiated properties of BMSc maintained in this study. Therefore, biodegradableCh/PEO nanofibers and thin film Ch scaffolds aresuitable modelsintissue engineeringandcell therapy.

REFERENCES

[Hosseinkhani H, HosseinkhaniM and Kobayashi H. Proliferation and differentiation of mesenchymal stem cells using self-assembled peptide amphiphile nanofibers. JBiomedical Materials. 2006; 1 : 8-15.

[Kazemnejad S, Allameh A, Soleimani M, Ahmad G, Mohammadi Y, Amirizadeh N, et al. Development of a novel three-dimensional biocompatible nanofibrous scaffold for the expansion and hepatogenic differentiation of human bone marrow mesenchymal stem cells. J Biotechnology. 2007; 4(3):201-211.

[Yarin AL, Koombhongse S, Reneker DH. Bending instability inelectrospinning of nanofibrous. J Appl Phys 2001;89:3018–26.

[Jayaraman K, Kotaki M, Zhang YZ, Mo XM, Ramakrishna S.Recent advances in polymer nanofibers. J Nanosci Nanotechnol2004;4:52–65.

[Martinová L and Lubasová D. Electrospun Chitosan Based Nanofibrous. RJTA 2008; 2: 72-79.

[Berger J, Reist M, Mayer JM, Felt O, Gurny R. Structure andinteractions in Chitosanhydrogels formed by complexation oraggregation for biomedical applications. Eur J Pharm Biopharm2004;57:35–52.

[Zhang Y, Zhang MQ. Calcium phosphate/ Chitosancompositescaffolds for controlled in vitro antibiotic drug release. J BiomedMater Res 2002;62:378–86.

[Zhang Y, Zhang MQ. Three-dimensional macroporous calciumphosphate bioceramics with nested Chitosansponges for load-bearing bone implants. J Biomed Mater Res 2002;61:1–8.

[Gutowska A, Jeong B, Jasionowski M. Injectable gels for tissueengineering. Anat Rec 2001;263:342–9.

[Bhattarai N, Edmondson D, Veiseh O. Electrospun chitosan-based nanofibrous and their cellular compatibility. J Biomaterials. 2005; 67: 35-42.

[Khora E, Yong L. Implantable applications of chitin and chitosan. J Biomaterials. 2003; 24: 2339–2349.

[Pittenger MF, Mackay AM, Beck SC, et al. Multilineage poten-tial of adult human mesenchymal stem cells. J Science. 1999;284:143-7.

[Orlic D, Kajstura J, Chimenti S, et al. Bone marrow cells regenerate infarcted myocardium. Nature 2001;410:701- 5.

[Yoon YS, Lee N, Scadova H. Myocardial regeneration with bone-marrow-derived stem cells. Biol Cell 2005;97:253- 263.

[Chen X, Katakowski M, Li Y, et al. Human bone marrow stromal cell cultures conditioned by traumatic brain tissue extracts: growth factor production. J Neurosci Res 2002;69:687-91.

[Chopp M, Li Y. Treatment of neural injury with marrow stromal cells. Lancet Neurol 2002;1:92-100.

[Orlic D, Kajstura J, Chimenti S, et al. Mobilized bone marrow cells repair the infarcted heart, improving function and survival. Proc Natl Acad Sci USA 2001;98:103 - 44.

[Woodbury D, Schwarz EJ, Prockop DJ, Black IB. Adult rat and human bone marrow stromal cells differentiate into neurons. J Neurosci Res 2000;61:364-70.

[Kaka GHR, Tiraihi T, Kheradman J, Azizzadeh AR. A Study on in-vitro Transdifferentiation of Rat Bone Marrow Stromal Cells into Neuroepithelial-Like Cells. IRCMJ 2009; 11: 133-139.

[Mingyu C, Jinguang D, Yang F, Yandao G, Nanming Z, Zhang X. Study of physical properties and nerve cell affinity of composite films from Chitosanand gelatin solutions.Biomaterials. 2003; 24: 2871–2880.

[GHR, Tiraihi T, Kheradman J, Azizzadeh AR. In Vitro Differentiation of Bone Marrow Stromal C ells into Oligodendrocyte-like C ells Using Triiodothyronine as Inducer.Journal of Neuroscience 2012; 1–11.

[Bhattarai SR, Bhattarai N, Yi HK, Hwang PH, Cha DI, KimHY. Novel biodegradable electrospun membrane: scaffold fortissue engineering. Biomaterials 2004;25:2595–602.

[Mengyan Li, Guob Yi, Yen Wei , Alan G. Diarmid M , Peter I. Electrospining polyaniline-contained gelatin nanofibers for tissue engineering applications. Biomaterials 2006; 27: 2705 – 2715.

[Dezawa M, Takahashi I, Esaki M, Takano M, Sawada H. Sciatic nerve regeneration in rats induced by transplantation of invitro differentiated bone marrow stromal cells. Eur J Neurosci.
2001;14:1771–1776

[Phinney DG, Kopen G, Isaacson RL, Prockop DJ. Plastic adherent stromal cells from the bone marrow of commonly usedstrains of inbred mice: variations in yield, growth, and differentiation. J Cell Biochem. 1999;72:570–85.

[Kopen GC, Prockop DJ, Phinney DG. Marrow stromal cellsmigrate throughout forebrain and cerebellum, and they differentiate into astrocytes after injection into neonatal mouse brains.Proc Natl Acad Sci USA1999;96:10711–10716.

[Lu L, Chen X, Zhang CW, Yang WL, Wu YJ, Sun L, Bai LM,Gu XS, Ahmed S, Dawe GS, Xiao ZC. orphological andfunctional characterization of predifferentiation of myelinatingglia-like cells from human bone marrow stromal cells throughactivation of F3/Notch signaling in mouse retina. Stem Cells.2008;26:580-90.

[Zhao LR, Duan WM, Reyes M, Keene CD, Verfaillie CM, Low WC. Human bone marrow stem cells exhibit neural phenotypes and ameliorate neurological deficits after grafting into the ischemic brain of rats. Exp Neurol. 2002 Mar;174(1):11-20.

[Bossolasco P, Cova L, Calzarossa C, Rimoldi SG, Borsotti C, Deliliers GL, Silani V, Soligo D, Polli E. Neuro-glial differentiation of human bone marrow stem cells in vitro. Exp Neurol. 2005 Jun;193(2):312-25.

[Lamoury FM, Croitoru-Lamoury J, Brew BJ. Undifferentiated mouse mesenchymal stem cells spontaneously express neural and stem cell markers Oct-4 and Rex-1. Cytotherapy. 2006; 210(2):228-42.