J. of Applied Tissue Engineering
December 2014; 1(1): 22-25

Evaluation the cytotoxic/viability effect of gelatin/bioactive glass conduits by MTT assay

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Received: 5 September 2013 / Accepted: 15 February 2014

Abstract:

Peripheral nerve injury can successfully heal,if an appropriate environment and route be provided.This study was designed to develop a novel gelatin/ bioactive glass conduits using freeze-drying technique. The gelatin/ bioactive glass conduits characterized using Fourier transform infrared spectroscopy (FTIR).The surface morphology of the Nanocompositewere investigated through scanning electron microscopy (SEM).Cytotoxicity Evaluation show that the CHO cells had attached and proliferated in the surroundings of the conduit. Biocomptibilitywas assessed by MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenylte-2H-tetrazolium bromide) assay which showed that gelatin/bioactive glass conduits had good cytocompatibilit.

Key Words: bioactive glass, Conduit,Cytotoxicity effects, tissue engineering.

IntroductionPeripheral nerves are exposed to physical injuries usually caused by trauma that may lead to a significant loss of sensory or motor functions and is considered as a serious health problem for societies today.This condition requires the use of nerve grafts and peripheral nerve substitutes to support the healing process.Autografts have been considered as the gold standard for peripheral nerve repair due to their obvious properties; however there are restrictions on autografts use such as limitations in availability of donor site,the sensation loss of donor area and donor site morbidity.

Conduits as a nerve growth structure have been developed to improve nerve gap regeneration.Tissue engineering,combining the biological knowledge, materials and the,is a contemporary approach to repair and rebuild the lost tissues and organs. Fabrication of porous gelatin scaffolds using a freeze-drying methodis also an effective method, due to advantage of this technique.

Gelatin, is a partial derivative of collagen, and has a wide range of uses in the pharmaceutical food and cosmetic industries[1].For applications as nerve tissue healing,gelatin was used as a polymer to increase the flexibility of the scaffold[2, 3].However, it has a poor mechanical property[1, 4]. Bioceramicsare often used in combination with biodegradable polymers to achieve the best possible mechanical and biological performance.Thecharacteristic ofbioactive glasses (BG) include; excellent bioactivity,ability to deliver cells, and controllable biodegradability[5]. These advantages make bioactive glasses a promising scaffold material for tissue engineering.In the present study, we investigated the suitability of a composite nerve conduit for peripheral nerve regeneration fabricated with bioglass and gelatin utilizing freeze drying technique.

Materials and methods

Conduits fabrication

At firsta homogeneous aqueous solution of microbiology-grade Gelatin (GEL) (10% weight per volume, w/v) was prepared and BG Nanopowderwas added to obtain a GEL (70)/BG (30) weight composition. After homogenization through stirring,special mandrelswere dipped in solutionseveral times. To produce porous structures, the mandrelswas transferred to a freeze dryer at -57° C and 0.05 mbar for 24h in order to produce a 3D porous structure through sublimation to form a gelatin network matrix on the pore walls and the surface of conduits.The emerging conduits had the following dimensions:Internal diameter 1.6 mm. Next, nanocomposite was soaked in a cross-linking bath of glutaraldehyde (GA) solution of0.5% (w/v) for 24 hours to reduce biodegradation and enhance the biomechanical properties(fig.1)

Fig.1 conduits after cross linking

Fourier Transforms Infrared Spectroscopy (FTIR)

The Fourier Transform Infrared Spectroscopy (FTIR) was operated in the mid-infrared range from 400–4000 cm^{-1} in reflection mode. The FTIR spectrometer was used to characterize the presence of specific chemical groups in the functional groups of Nanocomposite conduits. For IR analysis, in first 1 mg of the powder samples were carefully mixed with 300 mg of KBr (infrared grade) and palletized under vacuum. Then the Pellets were analyzed at the scan speed of 120 scan min^{-1} with 4 cm^{-1} resolution.

Scanning electron microscopy (SEM)

Scanning electron microscope photomicrographs (SEM-Philips XL30) were used to measure the average pore size of the modeled conduits. The Nanocomposite samples was coated with gold using a (EMITECH K450X, England) Sputter before examination under scanning electron microscope that operated at the acceleration voltage of 15 kV.By using SEM, the pore size of the cross-section of the samples was observed.

Cell Seeding on conduit

gelatin/ bioactive glass conduits were sterilized by ethylene oxide at 38°C and 65% relative humidity for 8 hours. After 24 h aeration in order to remove the residual ethylene oxide, the conduits were placed inside a standard 24-well-plate and finally with culture medium. For cytotoxicity evaluation, culture in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum(FBS) and streptomycin/penicillin 100 U/mL (1%).Chinese hamster ovary cells with a density of 4×10^5 cell/ml were added to the samples in PS plates and maintained in an incubator (37˚C, CO_2 5%) for 48h[6].The Nanocomposite conduits crosslinked with GA o.5% (w/v) were studied for this reason. The samples were kept in 100% ethanol for 15 min, and then visualized by light microscopy (Nikon Eclipse 50i)[7].

MTT detection of viable cells

The viability and proliferation of layered BG conduits were determined by 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay[28].Cytotoxicity effects of conduits were investigated on Miapaca-2 cell lines (purchased from the Pasteur Institute, Iran).The cells were plated in 96-well culture plates at 1.7x 104 cell/well. They were cultured in RPMI-1640 supplemented with 10 % FBS and 1% PS in 5% CO_2 at 37°C. After 72h, mediums were removed and 100 µL of fresh medium and 13 µLof MTT solutions (5 µg/ mL, diluted with RPMI 1640 without phenol red) were added to each well. Incubation was allowed for another 4h in dark at 37°C. Mediums were removed and 100 µL/ well DMSO (dimethyl sulfoxide, Sigma, Aldrich, Germany) was added to dissolve formazan crystals. Wells were finally read at 540 nm on an ELISA plate reader (Tecan Sunrise TM) and percentage of viability calculated.The well without conduit was used as a negative control and cell viability was defined as 100% for the MTT assay control. Each test was repeated three times.

Results

FTIR analysis

The BG conduits also represent the FTIR spectrum of the Nanocomposite that exhibited a number of new characteristic spectral bands, the most characteristics of which were protein spectrums such as: N–H bending vibration at 1260 cm^{-1} for the amide III, N–H bending vibration at 1560 cm^{-1} for the amide II, C=O stretching vibration at 1670 cm^{-1} for the amide I, C–H bending vibration at 2952 cm^{-1} for the amide B and band at 3570 cm^{-1} indicating the presence of O–H groups, while the characteristic spectral bands for BG was present in the Spectrum for Nanocompositas well(Fig.2)

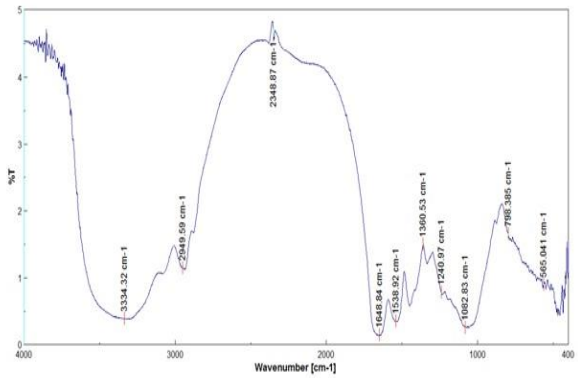

Fig.2FT-IR spectroscopy

SEM observations

The SEM micrograph images showed the porous structure of the conduit (Fig. 3).The sponge-like inner layer of conduits could permit the exchange of Feeding and fluids, and Ability a great space for the storage of the released Nerve growth.

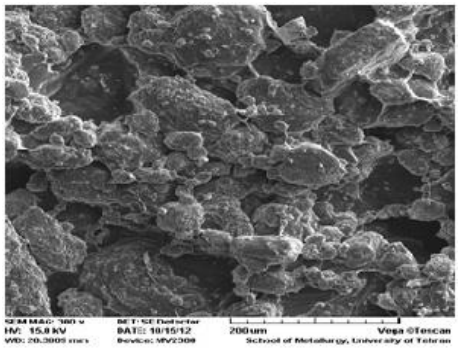

Fig3.SEM image of the porous nanocomposite conduit, inner layer of conduit wall (300×)

Figure 4 shows the pictures taken from seed cells on conduit specimens. The biocompatibility of the conduits, which was cross linked with 0.5 % GA, was assessed for cellular attachment, spreading and finally developing filopodias. After 3 days of culture, the CHO cells had attached and proliferated in the surroundings of the conduit

MTT detection of viable cells

MTT tests showed that the cell viability of Miapaca-2 cultured in the conduit extract was not significantly different from that in plain medium of cells during 72 h. This result clearly suggested that the fabricated conduits were nontoxic and posed as good candidates to be used as nerve conduits(Fig.5).

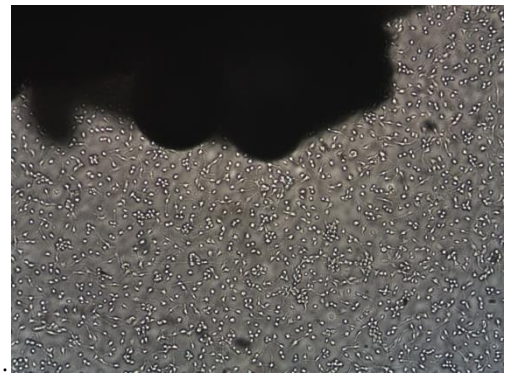

Fig4. Chinesehamsterovarycells cultured on the produced conduits cross linked with 0.5% GA.

The results obtained from the test and control after 72 h showed no significant cytotoxicity effects (Pv≤0.05).

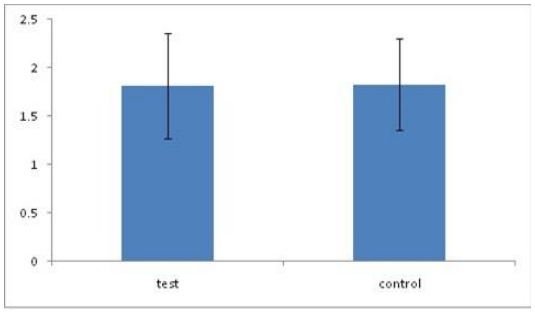

Fig.5MTT analysis results after 72 hours. Groups Test and control indicate disc and negative control Samples, respectively. No significant differences were observed between the two groups test group and control group.

Discussion

The gelatin/ bioactive glass conduits were prepared from a mixture of aqueous gelatin solution with Bioactiveglass synthesized through the Sol/Gel method by freeze drying techniques as a bioactive Nanocompositeconduit.

FTIR microscopy has been proofed to be a powerful tool to characterize the presence of various chemical groups. In previous studies for gelatin and hydroxyapatite that the first one at about 1,359 cm^{-1} indicates the formation of the chemical bond between carboxyl groups from gelatin and Ca^{2+} ions from the BG[8, 9].The second bond at 2.349cm^{-1} appeared after crosslinking of gelatin with GA as mentioned former by Azami, et al[8].

The SEM micrograph images showed the porous structure in the dense outer layer and the inner layer of the our conduit the is conforming with results SEM test Yumin Yang et al[10].

Our data obtained from MTT analysis showed no cytotoxic

effects on viability and proliferation properties of cells during 72 hours.Son˘aJantova´and Colleagues reported that the bioglassscaffold just a slight cytotoxicity and comfortablebiocompatibility[11]. Therefore their data confirmed our data in this regard.

ConclusionsAccording to our findings gelatin/ bioactive glassconduit,as a biocompatible novel biomaterial, could be a suitable candidate to be used for peripheral nerve regeneration.

Acknowledgments

The authors appreciate the Baqiyatallah University of Medical Sciences, Tehran, Iran for their financial support to perform this project.

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