In vitro evaluation of novel Zeolite-hydroxyapatite blended scaffold for dental tissue engineering

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Abstract

Main purpose of tissue engineering is creating appropriate conditions for the regeneration of tissues. Dental pulp-derived stem cells due to differentiation capacity and angiogenic properties have potential to regenerate dental pulp tissue. In the current experimental study poly caprolactone and poly L-lactic acid were synthesized by ring-opening polymerization method.

The nano-hydroxyapatite and Zeolite were obtained by hydrothermal method. Morphological features and crystals properties of nHA and Zeolite were studied by X-ray diffraction. Nanofibers were fabricated using electrospinning method and investigated by FT-IR spectroscopy. DPSCs obtained from human source and proliferation and viability of them on electrospun scaffolds were evaluated by MTT assay. Also, the adhesion and proliferation of hDPSCs were investigated by SEM. The results showed that hDPSCs have the most viability and proliferation on the 1 st , 7 th , 14 th days on PCL-PLA/Zeolite scaffolds and maximum on the 3 rd day on PCL-PLA/nHA scaffolds. On the days of 7 th and 14 th , cell growth on scaffolds containing both nHA and Zeolite is better than sample that nHA is used alone with PCL-PLA.

Briefly, by these results can be understand that Zeolite is a good agent in bone and tooth tissue engineering applications. More studies requires to investigate Zeolite effect on scaffold properties.

1. Introduction

Tissue regeneration (TE) in dental region is in high requisition because of difficulties such as trauma, after-cancer surgery, skeletal system disease, periodontal disease, and congenital disorders [1]. TE is interdisciplinary science that plays the main role in the regeneration of the lost organs. Regenerative medicine is emerging fields such as tissue engineering, material science, cell and molecular biology, sometimes used via collaborates these fields with biotechnologies to provide tissue regeneration [2]. The main purpose of tissue engineering is to create the necessary conditions for tissue regeneration. The most important components of this group are: scaffolds, signal molecules, and stem cells [3, 4]. Desirable scaffold properties include: Three-dimensional (3D) structure with suitable mechanical properties, appropriate cellular activities (cell adhesion, proliferation and differentiation), optimized porosity (size, porosity shape, interconnectivity) to allow cellular nutrition and tissue formation, appropriate biodegradability rate according to new tissue formation, use of repeatable techniques for controlled shapes or sizes [5, 6]. Due to high surface-to-volume ratio of nano fibers, they are good candidate for scaffold manufacturing in the first step of tissue engineering [7]. Electrospinning is a simple and inexpensive technique by which aligned or random micro/nanofibers can be fabricated.

Easy training and utilization, commercial applications, easy fiber functionalization, and relatively low startup cost, producing thin layer of fibers that have large surface area and providing suitable mechanical properties are advantages of electrospinning [8, 9].

The size of obtained fibers by this method is in the range of nanometers to micrometers. So, it can mimic physico-chemical properties of natural bone Extracellular Matrix (ECM) [10].

In electrospining technique, proper viscoelastic solution is made of polymer or ceramic with suitable solvent. Homogenized solution transferred to the syringe with suitable tip of nozzle diameter. high electric field is applied to solution and Tylor cone is formed at the tip of nozzle. Due to electrical potential difference between collector and tip of nozzle, fibers (micro and nano) spined on the collector. the solvent evaporates and dry solid fibers are formed on the collector [11].

An effective parameters in fibers quality are divided into three categories: Solution-Dependent Parameters, Environment-Dependent Parameters and Device-dependent parameters.

Nanofibers are worthy candidates for tooth regeneration because of their semblance to ECM and acceptable porosity so that cells can be attached, differentiated and proliferated [12, 13].

The 3D structures with appropriate interconnectivity and porosities help cellular motility, adherent, reorganization and comfort transport of nutrients in to the scaffold for cell use and transfer waste material out of it [14].

Fibers diameter achieved from different fiber production techniques is smaller than 10 μm while these fibers is larger than required size for ECM (50–500 nm). For this reason, different techniques such as: 3D printing, electrospinning, self assembly, phase sepration and … were designed for creating 3D structures that simulate the ECM geometry [14, 15]. Electrospinning is one of the most common and popular techniques that used for preparation intended nanofibers and due to it’s properties, is taken into consideration [16].

Stem cells were quickly adopted and these can colonization of self-renewable progenitor cells to constitute one or more cell types due to they are considered as key elements of tissue engineering.

Currently, pulpal structures of primary and permanent teeth, periodontal ligaments, apical papilla, and dental follicles are stem cell contents from dental structures have been examined by various research groups [4]. One of the available and promising sources for mesenchymal stem cells is DPSCs. Dental pulp is the soft connective tissue that dentine surrounded it. It has unique features such as: dentine generation, avascular dentine Nutrition, nerves support. In recent years, many studies have investigated the use of hDPSCs for bone and tooth tissue engineering [17, 18].

In recent years, extensive research has been done on bone and tooth regeneration by nanomaterials and polymers. Among the polymers used, the most important rigid polymers are PCL, Poly Lactic-co-Glycolic Acid (PLGA) and polyglycolic acid (PGA), as well as soft polymers including collagen, fibrin, alginate, hyaluronic acid, and silk [19]. Different bioactive ceramics include calcium phosphates (hydroxyapatite, tricalcium phosphate, etc.), bioactive glass, silica-based biomaterials such as Zeolite, and silicate-based substances (baghdadite, hardystonite, etc.). These bioceramics are commonly added to biopolymesrs to prouduce suitable composite with osteoconductive properties for bone regeneration [20-22]. Materials that used to prepare scaffolds should be biocompatible, biodegradable (degradation rate is important), degradation products have no toxic effect on cells and should have FDA approve for clinical studies [23].

PCL is a synthetic polyester polymer that can be degrade by hydrolysis of its ester linkages in physiological conditions, this polymer is consist of low melting point (Tm = 60 °C). PLA is one of rigid biomaterials and aliphatic synthetic polyester polymer. Changing polymer ratio, molecular weight, crystallinity can effect on viscosity, porosity, structure and degradation rate of PLA [24]. PCL and PLA are biodegradable, biocompatible with low antigenicity and toxicity polymer. Both of these have been approved by FDA and widely can be used for medical applications as TE investigation [24]. Calcium phosphates are most similar to bone tissue and they are biocompatible. One of the calcium phosphates that has many applications in medicine is Hydroxyapatite (HA) [Ca_{10} ($P0_4$)$_6$ (OH)$_2$] [25, 26]. Due to calcium phosphates and hydroxyapatite similarity to bone tissue in terms of chemical composition, the lack of inflation and inflammatory reaction and ability to produce bone cells, they have been noticed in bone scaffold design and it uses as bone replacement material. Nanoscaled hydroxyapatite has excellent bone integration ability and biocompatibility [23]. nHA is widely used bioactive ceramic in dentistry and bone replacement applications that helps tissue repair or regeneration. It allows special biological reactions in intersection of tissue and implant [23, 27, 28]. Zeolites are mineral combinations including Na, K, and Ca with porous and hydrophilic structure. Zeolite (source of silica) causes effective bonding between designed scaffold and damaged tissue [29-31]. owing to its low cost, lack of toxicity, large surface area, rapid diffusion characteristics, adjustable porosity, and high mechanical strength over amorphous porous silica; Zeolite is a good candidate for bone and tooth TE applications [32]. Semi-permanent antibacterial properties of Zeolite, makes it suitable used for dental applications. Zeolite powder has been also used to improve scaffold's physical, mechanical, and biological properties [33]. Advantages of Zeolites includes: tailorable surface groups, controlled hydrophilic/hydrophobic properties and different methods for alterability of acidic/basic nature of Zeolites. It can be used for antibacterial effect, antitumor, anti-thrombotic agents, homeostatic, drug carriers and bone regeneration [34, 35]. Composite scaffolds that contain of ceramic/polymer, collect components advantages and minimize disadvantages of each component. Composite materials, in particular, ceramic/polymer bio composite scaffolds provide acceptable mechanical properties and appropriate osteoconductivity for bone and tooth TE [6, 36]. Bioactive composites composed of nHA and Zeolite are widely used in scaffold designing [20].

PCL-PLA nanofibers have poor hydrophilicity, smooth surface, low cell adhesion and migration; in order to improving properties, nHA and Zeolite were added to PCL-PLA in nanofiber producing process [37]. nHA and Zeolite existence in scaffold structure has positive effect on osteoconductivity and osteoinductivity of scaffold and improve cell behavior of PCL-PLA scaffolds. Poor hydrophilicity of PCL causes prolonged degradation rate of scaffolds and increased mechanical strength of them which are two an important factors in bone and tooth regeneration.

The aim of this study was to fabricate PCL-PLA, PCL-PLA/nHA, PCL-PLA/Zeolite and PCL-PLA/nHa/Zeolite nanofibers using the electrospinning method and investigate the proliferation difference of human DPSCs on 4 types of designed scaffolds.

2. Materials And Methods

2.1. Materials

DL-lactide and ε-caprolactone were purchased from Sigma–Aldrich (Co., Steinem, Germany) and recrystallized twice from ethyl acetate, and dried under high vacuum at room temperature before usage. Stannous 2-ethyl hexanoate (stannous octoate, $Sn(Oct)_2$) was purchased from Sigma-Aldrich (USA). Glutaraldehyde (25% aqueous solution) and all the solvents purchased from Merck Inc. MTT (3-(4,5-dimethylthiazol-2-yl)-2,5- diphenyltetrazolium bromide) was purchased from Sigma-Aldrich. Dulbecco Modified Eagle's Medium (DMEM), fetal bovine serum (FBS) and trypsin– EDTA were purchased from Gibco.

2.2. Synthesis of PLA

PLA was synthesized by ring-opening polymerization method. For this purpose, a certain amount of DL-lactide monomer was heated at 150 °C for 1 hour inside the balloon (With continuous flow of nitrogen atmosphere). Then, 0.1gr of $Sn(Oct)_2$ (as catalyst) was added to lactide while being stirred. After that, the temperature of reaction was decreased to 120 °C and kept constant at this temperature for 6 hour. In order to remove excess catalyst and unreacted monomers, the product was dissolved in dichloromethane and precipitated in cold diethyl ether.

2.3. Synthesis of PCL

PCL was synthesized by ring-opening polymerization of ε-caprolactone in the presence of stannous octoate as catalyst. A certain amount of Ɛ-caprolactone was heated at 150 °C in a two-necked, round-bottom flask (With continuous flow of nitrogen atmosphere). After that, 0.1gr of $Sn(Oct)_2$ was added to reaction. Then, the temperature of reaction decreased to 120 °C and kept constant at this temperature for 6 hours. Excess catalyst and unreacted monomers removed with dissolving in dichloromethane and transferred in a cold diethyl ether.

Both PCL and PLA were placed in to the avon vacuum for polymerization process completion.

nHydroxyapatite (nHA) and Zeolite powders were synthesized by hydrothermal method separately [38].

2.4. Designing and Fabricating of Nanofibrous Scaffolds by Electrospinning Method

Fibers were designed in 4 category: PCL-PLA, PCL-PLA/nHA, PCL-PLA/Zeolite and PCL-PLA/nHA/Zeolite. For all the samples, uniform solution of them was transferred in to the 5 mL plastic syringe with a metal needle tip (gauge 18). The process of the electrospinning for fabrication of nanofibers was shown in Figure 1. Electrospinning device settings (Fanavaran Nano-Meghyas, Iran) explained as follow: Drum speed: 130 rpm; Potential difference (Voltage): 20-25Kv; Temperature: 25 °C; Distance of collector and needle tip: 15 cm; Injection rate: 2ml/h.

2.4.1. Fabrication of PCL-PLA Nanofibers:

PCL-PLA with a molar ratio of 4:1 was dissolved in DCM: Methanol (4:1 v/v ratio) to prepare 15 wt% solution. The prepared solution was put in to container and be stirred for 24 hours at 25˚C temperature. Then, the electrospinning method was used for fabrication of PCL-PLA nanofibers.

2.4.2. Fabrication of PCL-PLA/nHA Nanofibers:

PCL-PLA with a molar ratio of 4:1 was dissolved in DCM: Methanol (4:1 v/v ratio) to prepare 15 wt% solution. Then, 0.1 gr HA nanoparticles were added to the PCL-PLA solution. The prepared mixture was stirred for 24 hours at 25˚C temperature. The electrospinning method was used for fabrication of PCL-PLA/nHA nanofibers.

2.4.3. Fabrication of PCL-PLA/Zeolite Nanofibers:

PCL-PLA with a molar ratio of 4:1 was dissolved in DCM: Methanol (4:1 v/v ratio) to prepare 15 wt% solution. After that, 0.1 gr Zeolite nanoparticles were added to co-polymers solution. The mixture of PCL-PLA solution and Zeolite was put in to container and be stirred for 24 hours at 25˚C temperature. The electrospinning method was used for fabrication of PCL-PLA/Zeolite nanofibers.

2.4.4. Fabrication of PCL-PLA/nHA/Zeolite Nanofibers:

PCL-PLA with a molar ratio of 4:1 was dissolved in DCM: Methanol (4:1 v/v ratio) to prepare 15 wt% solution. Then, 0.05 gr Zeolite nanoparticles and 0.05 gr HA nanoparticles were added to PCL-PLA solution and stirred for 24 hours at 25˚C temperature. The electrospinning method was used for fabrication of PCL-PLA/nHA/Zeolite nanofibers.

All the fabricated nanofibers dried for 3 days before cell culturing on them.

2.5. Fourier Transforms Infrared (FT-IR) Spectroscopy

The chemical structure of the prepared electrospun nanofibers was studied by FTIR spectrometer to identified functional groups of scaffolds (Equinox 55 LS 101, Bruker, Germany). The sample was mixed with potassium bromide and pressed to form a disk. The IR spectra of scaffolds were obtained in the range of 400 to 4000 cm^{-1}.

2.6. X-ray Diffraction (XRD) Analysis

XRD is an old and widely used technique for investigation of the crystalline structure. This technique was used to study the properties of crystal structure such as: network geometry, determination of crystalline phases, lattice defects, determining the crystal size and etc.

For this purpose, synthesized HA and Zeolite were studied by XRD technique to ensure the formation of respective phases and check size of synthesized particles. XRD analysis of nHA and Zeolite was done by

Bruker Discover 8 X-ray diffractometer (Germany) operated at 40 mA and 40 kV which was measured with Cu-Kα radiation in a 2θ range from 5° to 70° at 0.05°/s. The d-spacing (d) corresponding to the XRD peak was determined from Bragg's equation, $n\lambda = 2d\sin\theta$, where θ is the diffraction position, λ is the wavelength (which is 1.54 Å for a Cu target), and n is an integer.

2.7. Preparation of Scaffolds for Cell Culture Studies

The scaffolds with different combinations of materials including 4 groups: PLA- PCL, PLA- PCL/nHA, PLA- PCL/Zeolite and PLA- PCL/nHA/Zeolite was prepared for further cell compatibility studies. About 100 mg of each scaffolds placed in 12-well cell culture plates and dipped three times in a 70% ethanol solution for 20-30 minutes. Then, the ethanol was evaporated in the air and the scaffolds were rinsed three times with sterile PBS solution to remove the residual ethanol. In the next step, the scaffolds were incubated in a routine culture medium containing DMEM/10% FBS at 37 ºC for 48 h. Then, the scaffolds were seeded by DPSCs at a density of 5×105 cells/well using routine medium. The media refreshed every 2 days.

2.8. Dental Pulp Stem Cells (DPSCs) Preparation for Transforming on Scaffolds

In a previous study, the protocol of isolation and characterization of DPSCs has addressed by the authors [39]. In the current study, we investigated DPSCs behavior on PLA- PCL, PLA- PCL/nHA, PLA- PCL/Zeolite and PLA- PCL/nHA/Zeolite scaffolds in 4 groups to check the effects of using Zeolite on viability and proliferation of DPSCs.

2.9. Cytotoxicity Analysis by MTT Assay

MTT assay is a method for investigation of cell viability and proliferation. Scaffolds were studied in two cases: cellular and cell free to remove the background absorbance. For this purpose, DPSCs were seeded on electrospun nanofibers; cell viability and proliferation were investigated in 1st, 3rd, 7th and 14th day. In brief: at the determined times, plates was taken out of incubator, the old medium was removed and 500 µL of MTT solution (Sigma) (10mg of MTT powder dissolve in 5ml PBS) and 1500 µL of sample medium was added to each wells and incubated for 4 hours at 37 ºC. Then, medium of each well was removed and 500 µl of dimethyl sulfoxide (DMSO) (Sigma) was added. The MTT was reduced by the mitochondrial dehydrogenase of living cells and DMSO dissolved the purple formazan crystals that can be readable by Elisa Reader. The optical density (OD) of each well measured at a certain wavelength (absorbance at 570 nm) by Elisa Reader machine (Awareness Technologies Stat Fax 2100 Microplate Reader). The viability was calculated using the formula: V = (ODsample - ODblank/ODcontrol - ODblank)×100. Where the blank is cell free scaffolds measured OD.

2.10. Cell Adhesion by Scanning Electron Microscopy (SEM) investigation

For cell attachment investigation, PCL-PLA, PCL-PLA/nHA, PCL-PLA/Zeolite, PCL-PLA /nHA/Zeolite scaffolds were seeded with DPSCs. The culturing period for cell attachment on nanofibers and morphological observation was 14 days. Preparation of nanofibers for SEM studies includes the

following steps: At the determined time, scaffolds were rinsed twice with PBS, then cells on scaffolds fixed in 2.5% glutaraldehyde that diluted with PBS buffer (fixative solution), rinsed in PBS at 4 ºC for about 24 h, dehydrated in a series of ethanol, rinsed twice with PBS and finally dried at room temperature. In the next step scaffolds containing cells coated with nanometer-thick gold and observed by SEM (ChamScan MV2300).

3. Results And Discussion

3.1. FTIR Analysis

FTIR analysis was used to provide information about functional groups that existence in designed scaffolds structures. The chemical structures of the PCL-PLA (Fig. 3, a), PCL-PLA/Zeolite (Fig. 3, b), PCL-PLA/nHA (Fig. 3, c), and PCL-PLA/nHA/Zeolite (Fig. 3, d) were studied by FTIR spectroscopy. The characteristic peaks of PCL at 1168, 1238, 1297, 1760, 2850, 2950 cm^{-1} can be attributed to the C–O–C stretching vibration, asymmetric C–O–C stretching vibration, C–O and C–C stretching vibration, C=O stretching vibration, symmetric CH_2 stretching and asymmetric CH_2 stretching, respectively [40]. The peaks of PLA at 1089, 1185 cm^{-1} are distributed by the backbone ester group. About HA, the peak at 630 cm^{-1} was associated with the stretching vibration of the O–H bond. The absorption bands at 956, 1100 cm^{-1} accounts for asymmetric stretching vibration of the P–O of PO_4^{3-} group. The bands at 1420-1470 cm^{-1} belong to the stretching vibration of CO_3^{2-}[12]. The peak at the 3570 cm^{-1} indicates the stretching vibration band of O–H. About Zeolite, the band at 1100 cm-1 corresponded to the Si–O–Si. The peak at 1270 cm^{-1} was attributed to the asymmetric stretching vibration of the Si–Al–O group. The symmetric stretching vibration of Si–Al–O was observed at around 660 cm^{-1} [37].

Most of the characteristic peaks appeared in the spectra of the composites; The density of FTIR peaks were under the influence of each component mass ratio. Analysis of FTIR peaks showed that PCL, PLA, nHA, Zeolite were mixed well with each other and a homogeneous chemical structure was formed after nanofibers production [37].

3.2. XRD Analysis

nHA and Zeolite phase formation in prepared samples were investigated by XRD analysis. Comparison between standard peaks and synthesized by hydrothermal method showed that the obtained nHA and Zeolite peaks are belong to nHA and Zeolite respectively (Fig. 4).

3.3. SEM Images of Zeolite and nHA powders

nHA and Zeolite powders were synthesized by hydrothermal method separately. The morphology and size of the powders were determined by SEM images. SEM images represent the nanoscale size of the HA particles.

3.4. Cell Adhesion Study by SEM

Electrospinning is a popular technique for nanofiber production and can produce appropriate scaffolds for tissue engineering applications. To investigate designed scaffold properties from the aspects of nanofibers quality, scaffolds interaction with cells, scaffolds effect on cell behavior, SEM studies was done. FESEM images of DPSCs cell attachment on the PCL-PLA, PCL-PLA/nHA, PCL-PLA/Zeolite, and PCL-PLA/nHA/Zeolite scaffolds after 14 days of culture were shown in Figure 7. Images showed that fibers arrange in 3D structure with nano diameters and cells have good adherence with all of the nanofiber scaffolds. The cells integration on the PCL-PLA/Zeolite and PCL-PLA/nHA/Zeolite nanofibers was more than control group. Also, there was an excellent connection between cells and between cells and scaffold fibers. It can be said that nanofibers provide more surface area and porous structure that is necessary for DPSCs attachment and proliferation.

3.5. Proliferation and Viability of DPSCs on scaffolds

For evaluation of biocompatibility of scaffolds, MTT assay was done. DPSCs were seeded on to PCL-PLA, PCL-PLA/nHA, PCL-PLA/Zeolite, and PCL-PLA/nHA/Zeolite scaffolds and the cell proliferation on each scaffold was assessed on 1st, 3rd, 7th and 14th day after culture (Fig. 8). All the prepared scaffolds induced the proliferation of DPSCs. Our data indicated that mitochondrial activity of DPSCs is higher in each scaffolds over cells grown in routine culture flasks. Therefore, all four scaffolds showed excellent cytocompatibility for DPSCs. Addition of nHA to PCL-PLA improved the viability and proliferation of DPSCs (Fig. 8). Moreover, Zeolite enhanced the proliferation and consequently absorbance of both PCL-PLA/Zeolite and PCL-PLA/nHA/Zeolite scaffolds. DPSCs indicated the most viability and proliferation on the 1st, 7th, 14th days on PCL-PLA/Zeolite scaffolds and maximum on the 3rd day on PCL-PLA/nHA scaffolds. On the days of 7th and 14th, cell growth on scaffolds contain nHA and Zeolite is better than sample that nHA is used alone with PCL-PLA in nanofibers ($p<0.05$). Therefore, DPSCs preferred to attach to the scaffolds that contain Zeolite in comparison to scaffold with nHA. Zeolite has positive effect on DPSCs behavior. Cells on Zeolite-containing scaffolds showed much better adhesion, viability, proliferation; therefore, nanofibers contain Zeolite are good candidate for bone and dental applications.

3D structure that created by electrospinning technique provide acceptable space for DPSCs activity on nanofibrous scaffold with micro and nano porosity. Interestingly, nHA and Zeolite in the structure of fibers improved osteoconductivity of designed scaffolds and cell adhesion rate at the same time. Modified nanofibrous scaffold with nHA and Zeolite contains various potentials that can increase cell viability, cell attachment and proliferation of DPSCs in comparison with free nHA-Zeolite scaffolds.

3.6. Contact Angle Analysis

The static contact angle measurements results are shown in Fig. 9. The common model to analysis the contact angle on a surface is Young equation:

See Formula 1 in Supplemental Files

In Young equation, θ is the contact angle, *γsv* and *γsl* are surface energies for liquid-vapor and vapor-solid, respectively. *γlv* represents surface energies for solid-liquid interfaces.

It's assumed that water drop has contact with all of the nanofibers under it. As shown in the pictures, PCL-PLA scaffold was the most hydrophobic group of scaffolds in comparison to other groups with θ= 125.5°. The second hydrophobic group of scaffolds was PCL-PLA/Zeolite scaffold with a contact angle of 118°. Zeolite has decreased the contact angel of PCL-PLA scaffold. The most hydrophilic structure among the four groups was PCL-PLA/nHA with a contact angle of 98°. nHA has increased the hydrophilicity of PCL-PLA scaffold. PCL-PLA/nHA/Zeolite scaffold has contact angel between PCL-PLA/Zeolite and PCL-PLA/nHA.

Conclusion

The goal of tissue engineering is to design practical scaffolds for therapeutic purposes. Therefore, fabrication of nanofibers with acceptable features is an important issue. In the present study, scaffolds contain PCL, PLA, nHA, and Zeolite were fabricated by electrospinning method. Cell behavior was investigated on 4 groups of designed scaffolds. Zeolite contained scaffold played a positive role on DPSCs viability and cell adhesion; it seems that Zeolite can be an important agent in bone and tooth tissue engineering applications. SEM images showed that Zeolite-contained scaffolds has positive effect on cell attachment and improve cell behavior on basic scaffold.

More studies require investigating the effects of Zeolite on scaffold properties. It is essential to obtain the optimal percentage of applied Zeolite and nHA in the scaffold structure. Optimal size of powders play a decisive role in the scaffold's properties and cellular differentiation. Further studies in this area are suggested. For example: animal studies, bacterial tests, mechanical properties of the designed scaffolds and study on differentiation.

Declarations

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Authors' contributions

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Competing interests

The authors declare that they have no competing interests.

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Abbreviations

DPSCs: Dental pulp-derived Stem Cells; PCL: poly caprolactone; PLA: poly L-lactic acid; nHA: nano-hydroxyapatite; hDPSCs: DPSCs obtained from human source; TE: Tissue regeneration; 3D: Three-dimensional; ECM: Extracellular Matrix; PLGA: Poly Lactic-co-Glycolic Acid; PGA: polyglycolic acid; HA: Hydroxyapatite; DMEM: Dulbecco Modified Eagle's Medium; FBS: fetal bovine serum; FT-IR: Fourier Transforms Infrared Spectroscopy; XRD: X-ray Diffraction; OD: Optical Density.

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Figures

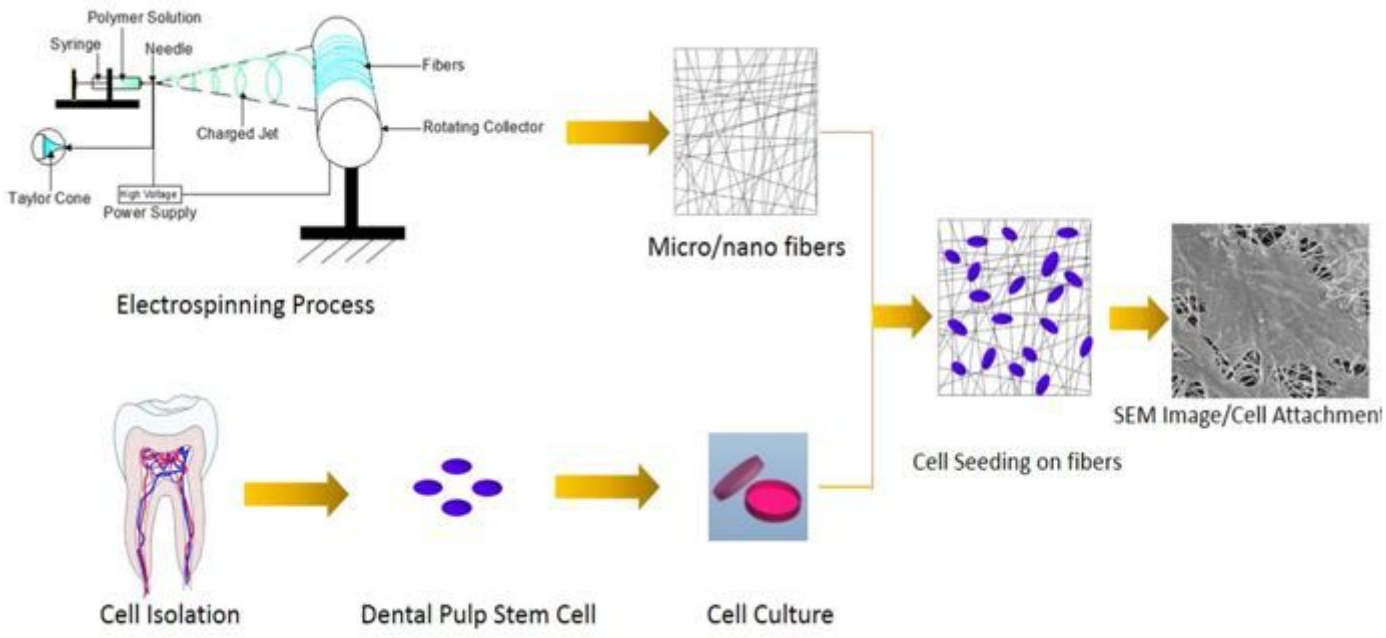

Figure 2

Schematic Abstract

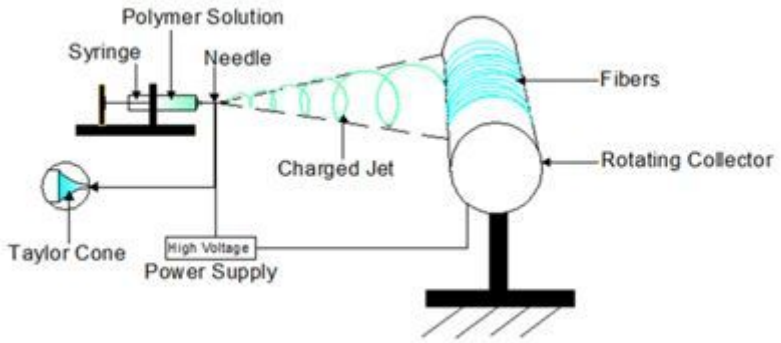

Figure 4

The electrospinning method for fabrication of nanofibers

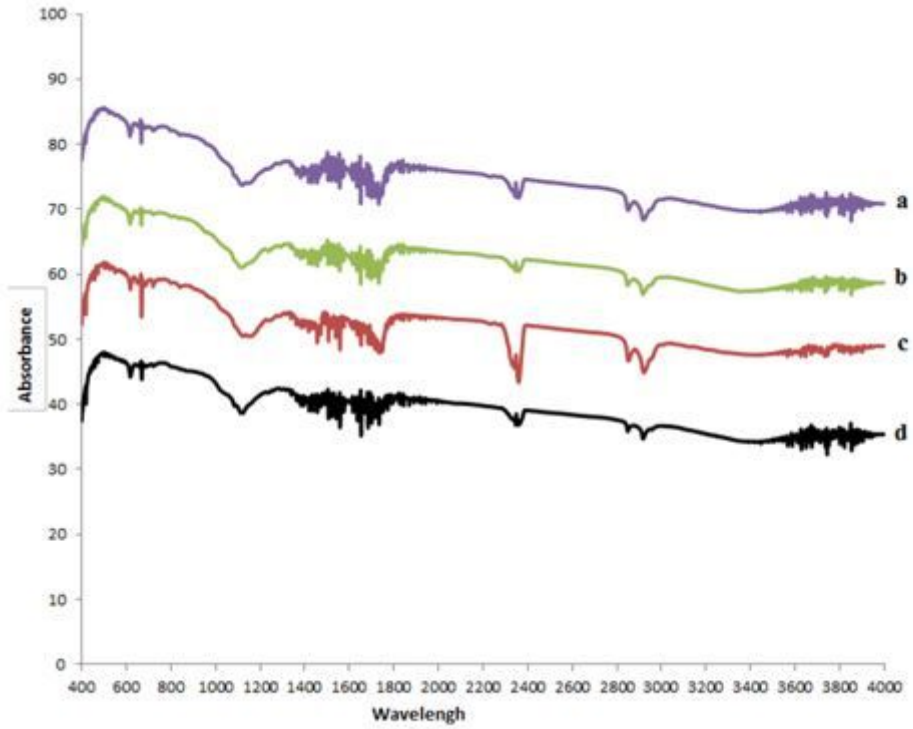

Figure 6

FTIR spectra of: a) PCL-PLA, b) PCL-PLA/Zeolite, c) PCL-PLA/nHA, and d) PCL PLA/nHA/Zeolite

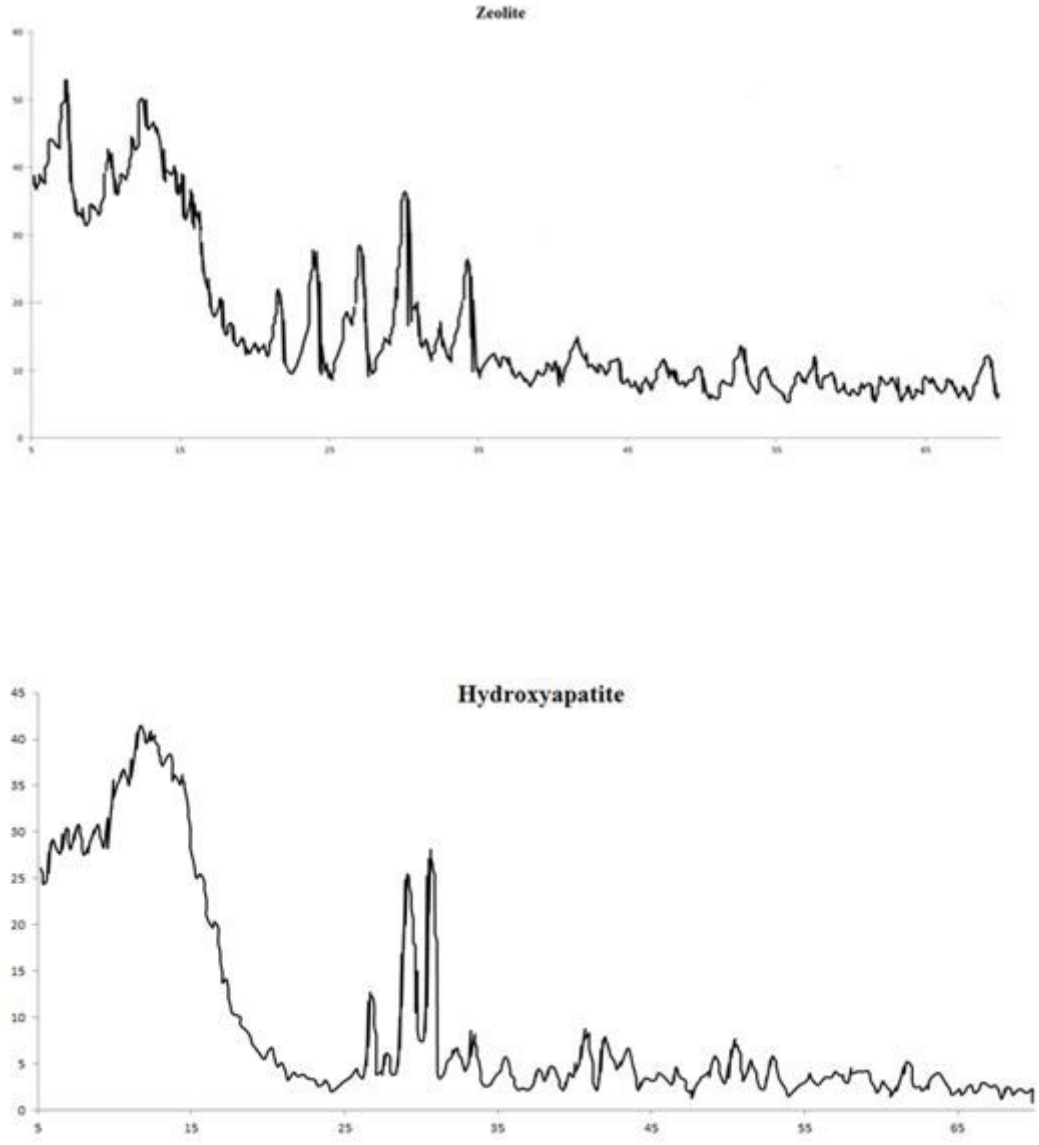

Figure 8

XRD pattern for the Zeolite and nHA samples

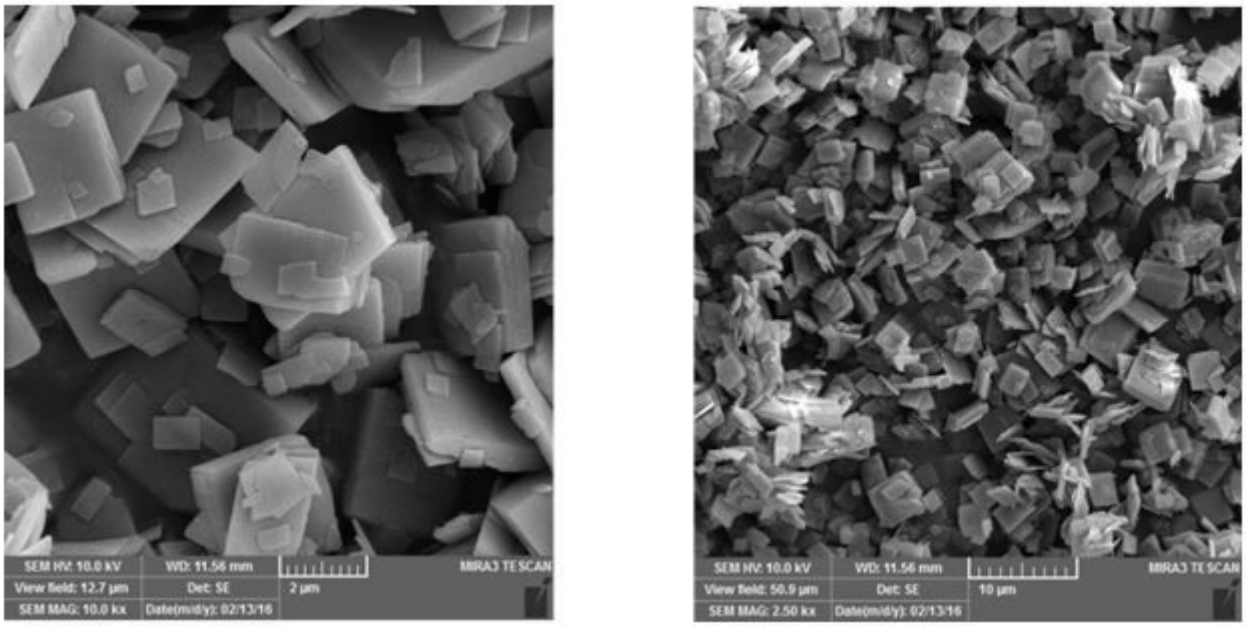

Figure 10

SEM Images of Zeolite powder

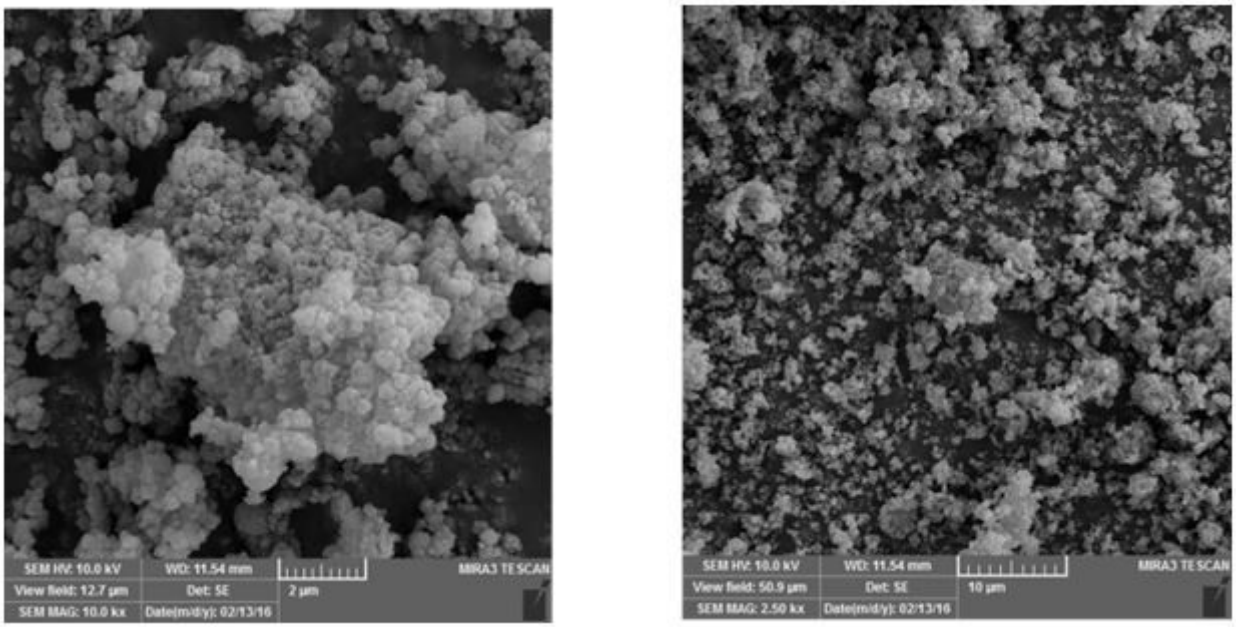

Figure 12

SEM Images of nHA powder

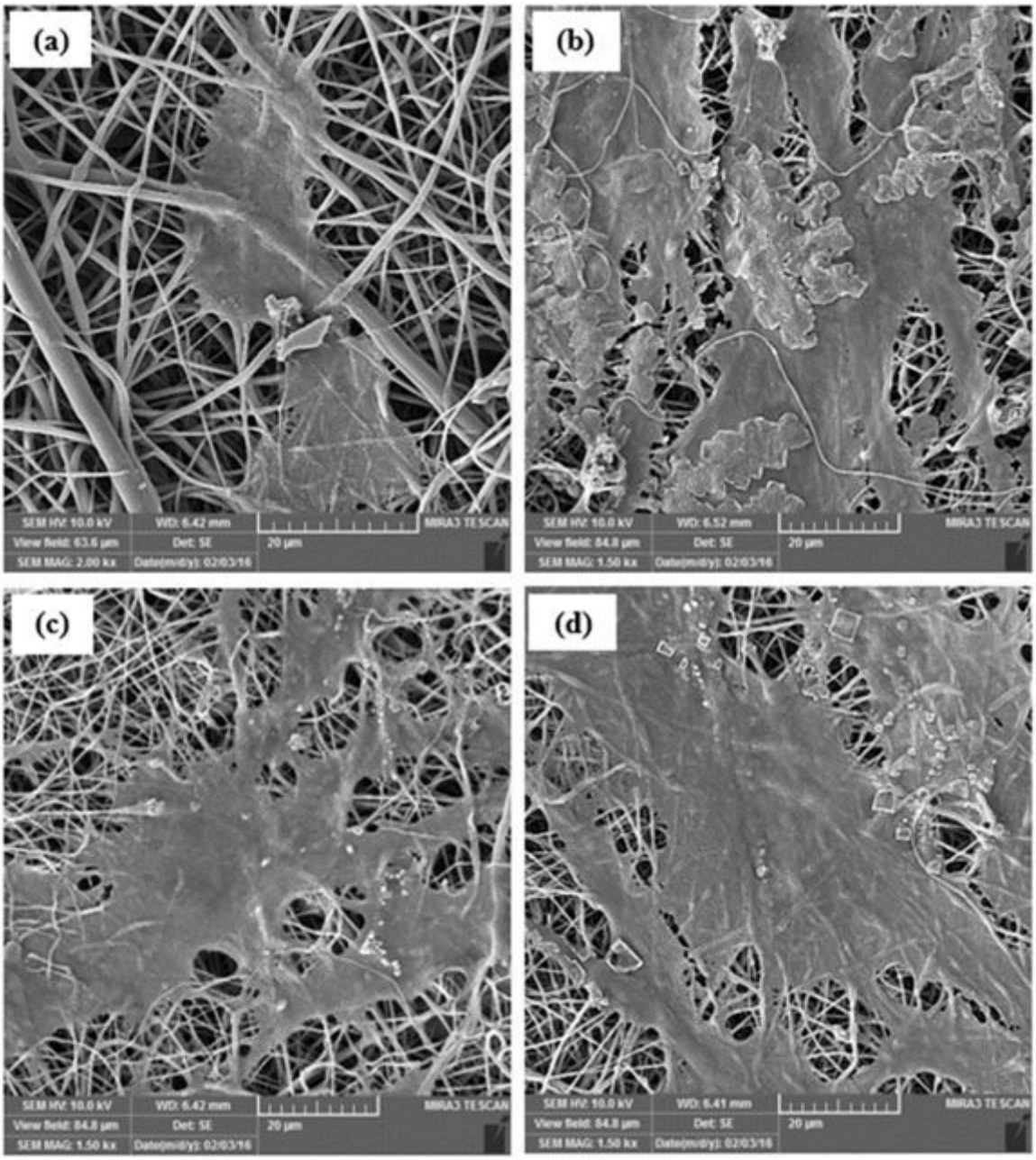

Figure 13

SEM images of DPCSc adhesion to designed nanofibers: a) PCL-PLA, b) PCL-PLA/Zeolite, c) PCL-PLA/nHA, and d) PCL-PLA/nHA/Zeolite

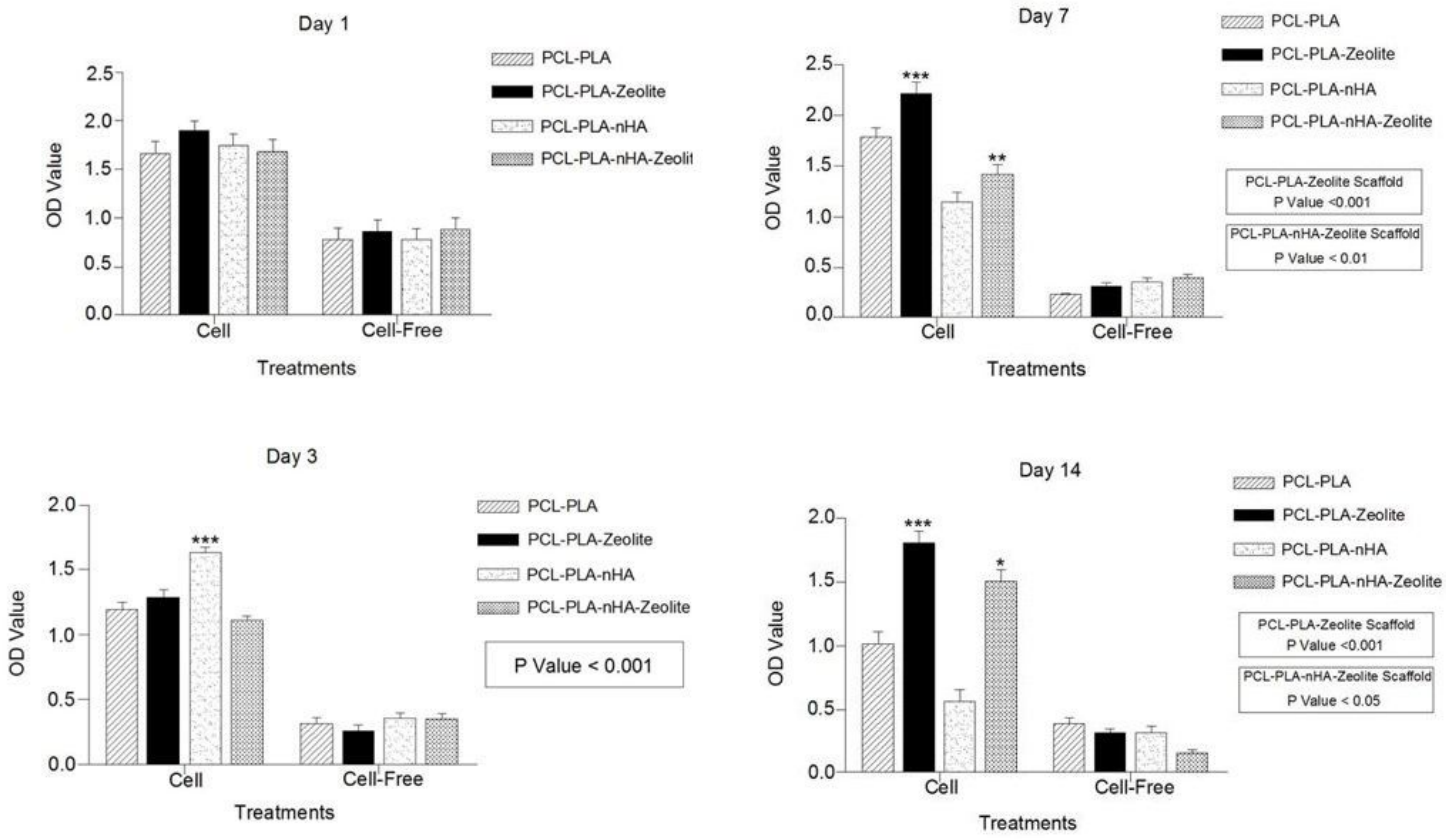

Figure 15

Cell viability study of DPSCs on the PCL-PLA, PCL-PLA/Zeolite, PCL-PLA/nHA, and PCL-PLA/nHA/Zeolite nanofibers

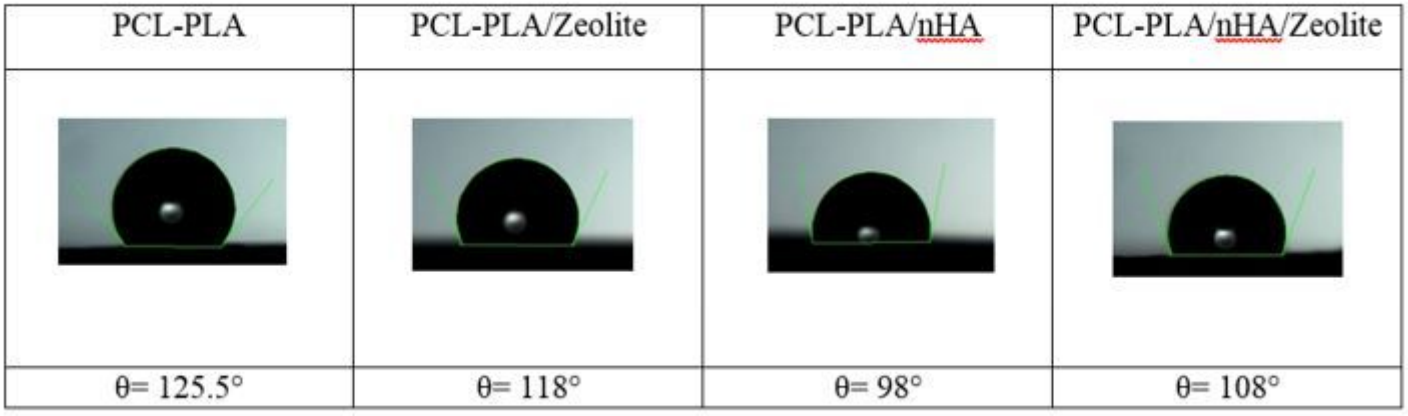

Figure 17

Micrograph of static contact angle

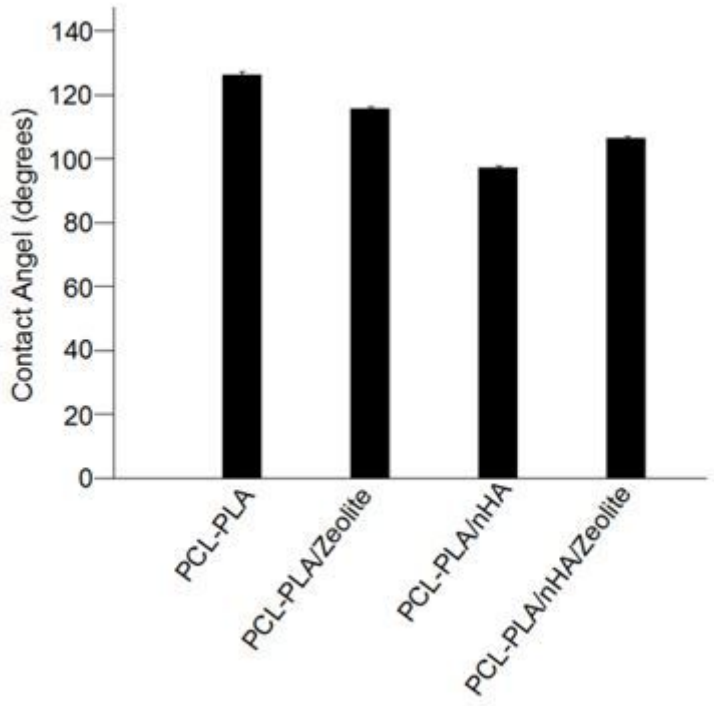

Figure 20

Contact angles of nanofibers

Supplementary Files

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- formula1.JPG