# Poly(lactic acid) (PLA) Nanofibers for Bone Tissue Engineering

Farnaz-sadat Fattahi, Akbar Khoddami*, and Ozan Avinc

*Abstract*- **Bone tissue engineering is the most promising therapeutic method to alleviate the fast-growing request for bone grafts in nonunion bone defects. It is founded on the use of implanted autologous cells or induced stem cells to form bone tissues on naturally derived or synthetic scaffolds. Nanofibers are being increasingly implemented in bone tissue engineering field as scaffolding materials to regenerate new bone tissues owing to their high surface area-to-volume ratio, high porosity with an interconnected pore structure and the suitable surface structure for cell attachment, proliferation, and differentiation. PLA biopolymer has captured the most attention and interest as a bone tissue engineering material since PLA is easily processable and degrades and disintegrates into natural metabolites while its degradation rate matches with the healing time of damaged human bone tissues. So, the potential of using PLA nanofibers in bone tissue engineering is a serious goal for scientists in novel investigations. This review gives detailed information about the recent developments and applications of PLA nanofibers as scaffolds for bone tissue regeneration.**

*Keywords*: **nanofiber, tissue engineering, poly(lactic acid), bone, scaffold**

## I. INTRODUCTION

Human organ and tissue failure inflicted by defects, accidents, injuries or other types of damages is one of the most problematic situations for human health. Unfortunately, there are more and more huge amounts of requests every year for a variety of biomedical implants to repair lost or diseased human tissues in consequence of world population growth [1,2]. Many various approaches like total artificial substitutes (artificial kidney, etc.), non-living processed tissues (heart valves, etc.) and transplantation have been applied to overcome these health problems [3]. However, for example in the case of organs transplantation, there are some handicaps such as the inadequacy of donor organs, the requirement of life long immune suppression and its critical complications [4,5]. Because of these reasons, tissue engineering has come to the forefront in the recent years [6,7]. Tissue engineering, as one of the most covetable treatment choices, comprises the elements of engineering, material and medical sciences and procures regenaration potential for virtually every tissue and organ of the human body [8,9]. Polymeric nano materials display unique characteristics which make them an important tool for tissue engineering field [10,11]. Nanoparticles for molecules delivery (drugs, growth factors, and DNA), nanofibres for tissue scaffolds, surface modifications of implantable materials or nanodevices (biosensors, etc.) are some of the important examples of nanomaterials used in biomedical applications and the mergence of these elements with tissue engineering is an important model of the immense potential of nanotechnology applied to regenerative medicine [12-14]. Nanofibers have been increasingly applied in biomedical fields such as scaffolding materials to regenerate new tissues because of their high surface area-to-volume ratio, high porosity with an interconnected pore structure, and the favorable surface structure for cell attachment, proliferation, and differentiation [1,15,16]. Different technologies come together to construct porous scaffolds including fiber bonding, porogen leaching, emulsion freeze drying, gas foaming, thermally induced phase separation, and electrospinning to regenerate the tissues/organs and also for controlled and targeted release of bioactive agents in tissue engineering implementations [17,18]. Poly(lactic acid) biopolymer has drawn the most attention among these polymers as a tissue engineering material in order to support bounce generation [15,19,20] because PLA is easily processable and degrades into natural metabolites while matching its degradation rate with the healing time of damaged human tissues [21,22]. PLA biopolymer can

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be used for bone regeneration not only due to its akin mechanical characteristics to the targeted tissue, but also owing to its good biological interactions with the host cells when it is implanted. Furtheremore some experimental pretreatments like UV/ozone and plasma can be used for developing PLA surface properties for bone regeneration [23,24]. Due to the widespread acceptance of the physical and chemical features of PLA nanofibres for bone tissue engineering field, this paper reviews some progressive studies and innovations in tissue engineering field. But before going into details about the usage of PLA nanofibers in bone tissue engineering field, firstly, brief introductory information will be given about bone tissue engineering and poly(lactic acid) for a more thorough understanding of the subject.

## II. BONE TISSUE ENGINEERING

Bone tissue engineering deals with the bone defects, neoplasia and cancers [25]. As a complex procedure, this method arranges bone cell migration, proliferation, differentiation and expedites bone matrix formation leading to shorter healing time in comparison with classical methods [15,26-28]. Bone is an extremely active and multifaceted tissue. Bone has a great hierarchical structural design which has several tasks, including holding the body, guarding organs, and storing nutrients (Fig. 1) [29-31].

Bone has a dense compressed shell named cortical bone and a porous core named spongiosa or trabecular bone [32]. It is composed of bone-forming cells, the osteoblasts, bone-resorbing cells, and the osteoclasts [33]. Bone extracellular matrix chiefly consists of both a non-mineralized organic material (specially type (I) collagen fibrils (Col)) and a mineralized inorganic material (composed of 4-nm-thick plate-like hydroxyapatite (HA, $Ca_{10}(PO_4)_6(OH)_2$) mineralites) [25,28,34,35]. The nanocomposite arrangement (tough and flexible Col fibers reinforced by HA crystals) is basic to the essential compressive strength and high fracture stiffness of the bone [15,36-39]. HA is the central part and the most important mineral component of natural bone in the type of calcium complexes [36,40,41]. Col is the main part of mammalian tissue, accounting for 30% of all proteins in the human body and 90% of the whole weight of bone ECM [30,36,42]. Bone tissue engineering deals with the bone defects, neoplasia and cancers. This process systematizes bone cell migration, proliferation, and differentiation and accelerates bone matrix formation leading to shorter curing time in comparison with traditional techniques [31,43].

### *A. Cell Sources for Bone Repair*

Human mesenchymal stem cells (hMSCs) are significant types of cells with self renewalability and immunomodulatory features. HMSCs (such as Human osteoblast cells and normal human osteoblasts (NHOst)) [44] have long been known for their potential in engineering bone grafts since they differentiate and generate bone

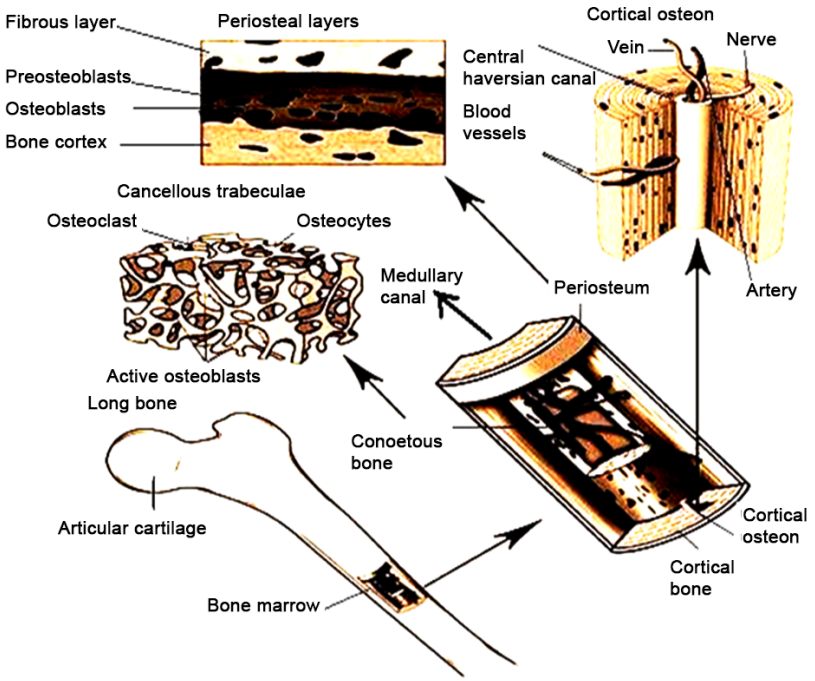



Fig. 1. Structure of bone [45].

during the natural bone development process [46,47]. Human mesenchymal stem cells have been segregated from numerous adult sources containing bone marrow, peripheral blood, umbilical cord blood, synovial membrane, deciduous teeth, dental pulp, amniotic fluid, adipose tissue, brain, skin, heart and kidneys [45,48]. The incorporation of hMSCs into biomaterials is a usual method for accelerated bone formation throughout defect repair and regeneration [49,50].

*B. Differentiation Factors Required for Bone Engineering*

Bone morphogenetic proteins (BMP) form a large sub-class of polypeptides whose functions are necessary throughout skeletal growth. Numerous bone morphogenetic proteins, including BMP-2, -4, -6, -7, -13, and -14 can enhance the synthesis of collagen type II in vitro [36].

## III. POLY(LACTIC ACID)

Various types of biomaterials are widely utilized in regenerative therapy and tissue engineering in bone because of their biocompatibility and biodegradability [16,51,52]. An ideal bone scaffold should be composed of biomaterials which closely mimic the structure and characteristics of natural bone extracellular matrix [29,30,53,54]. Both natural and synthetic polymers can be selected for manufacturing nanofiber scaffold in bone tissue engineering usage. The most commonly used biodegradable synthetic polymers for three-dimensional (3D) scaffolds in bone tissue engineering are saturated poly(α-hydroxy esters), [55-58] including poly(lactic acid) (PLA) and poly(glycolic acid) (PGA), as well as poly(lactic-*co*-glycolide) (PLGA) copolymers [59-61]. Beween these polymers, PLA has attracted the most attention as a bone tissue engineering material [5,62], because this biopolymer has some advantages over other materials used in this field that are:

- PLA can undergo scission in the human body [53].
- PLA degrades to monomeric units of lactic acid as a natural intermediate in carbohydrate metabolism [19, 63-65].
- PLA degradation rate matches with the healing time of damaged bone tissues [9,37,66].
- Thermal and mechanical properties of PLA are similar to those of bone tissues [67].
- PLA has nice biological interactions with the host bone cells when it is implanted [60,68-71].

PLA is a biodegradable, bioabsorbable, and biocompatible linear aliphatic thermoplastic polyester [20,68,72,73], produced from 100% renewable resources like corn, starch, wheat and rice [69,70,74]. It is a polymeric helix with an orthorhombic unit cell [75-77]. Lactic acid (2-hydroxypropionic acid, $CH_3$–CHOHCOOH) is the monomeric building block of PLA [64]. PLA possesses stereoisomers like poly(L-lactide) (PLLA), poly(D-lactide) (PDLA), and poly(DL-lactide) (PDLLA) [19,78-80]. PLLA has obtained a lot of interest due to its outstanding biocompatibility and mechanical features [64,81,82]. Nonetheless, its long degradation times together with the high crystalline nature of its fragments could result in inflammatory reactions in the body [83,84]. As earlier mentioned, PLA and PLLA have captured increasing attention as a bone tissue engineering material [85-87] in the forms of nanofibres, nanocomposites, nano paticles, microspheres, multilayer nanofiber fabrics, nanocomposite fibers and nanosheets [55,77].

## IV. PLA NANOCOMPOSITE FIBERS

It is clear that a polymer material cannot independently meet every practical requires of a scaffold for an optimized function in tissue engineering. A large number of synthetic and natural polymers are hydrophobic with different properties. Since ideal bone scaffold should have properties similar to natural tissues, to optimize the synthetic scaffold, a combination of two or more synthetic or natural polymers can be employed. As earlier mentioned, PLA biopolymer has captured increasing attention between other polyesters as a tissue engineering material. PLA nanofibres have an important share in such applications [88,89]. In spite of being biocompatible, clinical implementation of pure PLA nanofibres for bone regeneration is hindered by poor osteoconductivity [62]. Therefore, PLA nanofibres are often used in combination with other materials, such as hydroxyapatite (HA), an inorganic filler largely utilized in bone tissue engineering because of its non-toxicity, bioactivity and osteoconductivity, and resemblance to natural bone minerals. Ceramics and bioactive glass are opportunely modified to render PLA nanofibres more biomimetic and able to increase bone regeneration [90]. Therefore, in the next section of this review, the different implementations of constructs based on PLA nano fibers and nanocomposite fibers in the bone tissue engineering field will be highlighted and examined in detail.

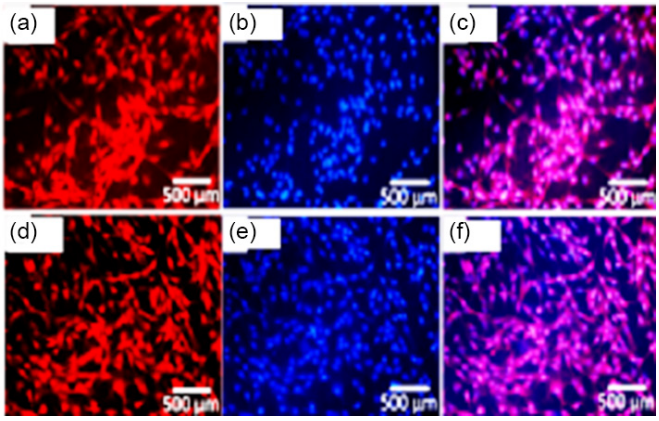
## V. APPLICATIONS OF PLA NANOFIBERS IN BONE TISSUE REGENERATION

For bone tissue engineering applications, PLA nanofibres have been utilized in a vast diversity of forms like multilayer nanofiber fabrics, porous scaffolds, nanofibres containing HA, porous scaffolds combined with growth factors (BMP family or omentum) which will be reported in detail in the following. In all cases after producing scaffolds, different types of bone cells were cultured on the scaffolds. Cell cultivation, alkaline phosphatase (ALP) assay, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium

bromide (MTT) assay and osteocalcin assay were examined to estimate cellular behavior (cellular attachment, viability, proliferation, migration, differentiation and proliferation) on the scaffolds. Furthermore, the study of the cells' morphology on the scaffolds was done by SEM, AFM, and fluorescence microscopy. Also the mechanical properties (Young's modulus, tensile strength) of scaffolds were analyzed and the osteogenesis characteristics of scaffolds were characterized by gene expression (Col (collagen) I, OPN (osteopontin), and OC (osteocalcin)). The result of these assessments will also be described briefly on the following pages.

In the newest research PLLA nanofibers were effectively decorated by ECM derived from osteoblastic cells via decellularization of MC3T3-E1 cells grown for two weeks. The outcomes revdeald that use of ECM on PLLA nanofibers developed mouse bone marrow stromal cell (mBMSC) adhesion, holding cell proliferation and supporting osteogenic differentiation of mBMSCs (Fig. 2) [91].

In another research in 2018 dexamethasone-loaded multi-layer PLLA composite nanofiber scaffolds were fabricated for bone tissue engineering. The scaffolds have suitable surface properties. The multi-layer scaffolds with the drug in the middle layer showed the best osteogenic proliferation and differentiation [92]. In the research in 2017, Chu-Jung Su *et al.* [93] fabricated PLA nanofibres via a modified electrospinning process, then mats were coated with chitosan/calcium silicate (CH/CS) mixture. Chitosan is a biomaterial with the advantages including high biocompatibility, low inflammatory responses from host, antibacterial features, and high biodegradability. On

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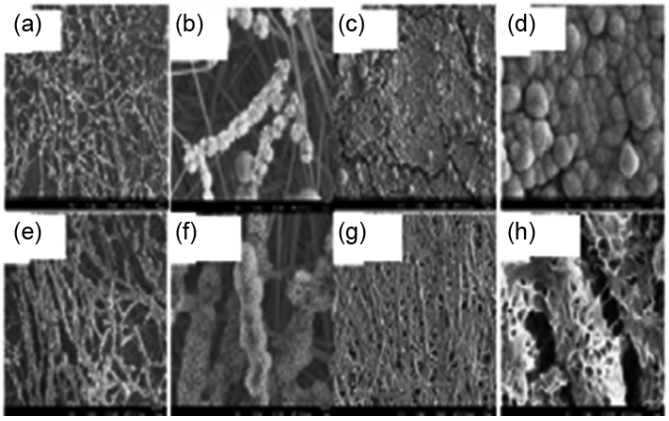


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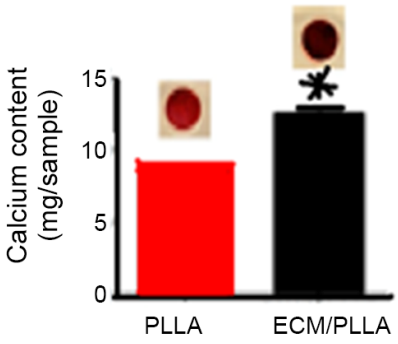



(3)

Fig. 2. (1) Immunostaining of actin, and nuclei showing mBMSC cells cultured on PLLA NF and ECM/PLLA NF, respectively: (a-c) actin staining, DAPI staining, and merged image of mBMSCs on PLLA NF at day 7, (d-f) actin staining, DAPI staining, and merged image of mBMSCs on ECM/PLLA NF at day 7; red: actin; blue: nuclei.

(2) Apatite coating formation on two types of PLLA nanofibers with different treatments: (a) apatite growth survey on PLLA NF at 24 h, (b) apatite growth zoom-in on PLLA NF at 24 h, (c) apatite growth survey on PLLA NF at 48 h, (d) apatite growth zoom-in on PLLA NF at 48 h, (e) apatite growth survey on ECM/PLLA NF at 24 h, and (f) apatite growth zoom-in on ECM/PLLA NF at 24 h, (g) apatite growth survey on ECM/PLLA NF at 48 h, (h) apatite growth zoom-in on ECM/PLLA NF at 48 h, after soaking in mSBF for different time periods.

(3) Calcium deposition on PLLA NF and ECM/PLLA NF after mBMSCs culture for 14 days: inserted images were Alizarin Red S staining of calcium. Calcium deposition was significantly higher on ECM/PLLA NF than on PLLA NF ($p < 0.05$) after 14 days.

* indicates significant differences [91].

the other hand, CS-based ceramics are typically utilized in hard tissue engineering because of higher biocompatibility, bioactivity, and biodegradability. The amalgamation of hMSCs into biomaterials is a common method for faster bone formation during regeneration [31]. In vitro hMSCs cultivation on pure PLA and CS/CH-containing mats verified that the addition of inorganic component would not be toxic and supports cell attachment and proliferation, which then results in better osteogenesis for bone tissue engineering [93]. A bicomponent scaffold with a core–shell construction that combines the benefits of PLA and chitosan (CS) was prepared via electrospinning escorted with automatic phase separation and crystallization. The mineralization of HA and culture results of preosteoblast (MC3T3-E1, Mouse Osteocalcin) cells on these scaffolds indicate that the outer CS component and rough nanoscale topography on the surface of the nanofibers balanced the hydrophilicity and hydrophobicity of the fibers, enhanced their mineralization ability, and made them more beneficial for the attachment and growth of bone cells [94]. In other research in 2017, Magiera *et al.* [5] manufactured PLA-based nanofibrous non-wovens which were modified utilizing two types of modifiers, namely, gelatin (GEL)-based nanofibres and carbon nanotubes (CNT). Mats being composed of PLA and GEL nanofibres (PLA/GEL), as well as CNT-modified PLA nanofibres with GEL nanofibres (PLA + CNT/GEL) were produced utilizing concurrent electrospinning method (co-ES) [91]. GEL is a biocompatible material derivatived from Col [95]. Researchers often use it in tissue engineering in the form of Col replacement for cell adhesion, migration, differentiation and proliferation [1,95,96]. CNTs can modify electrical and mechanical properties of PLA fibres obtained via co-ES for stimulating bone tissue formation. Normal human osteoblasts (NHOst) were cultured on the scaffolds. Morphology (fluorescence microscope) of NHOst cultured for three days in contact with fibrous scaffolds showed that cells covered homogeneously and thickly the materials' outsides. Their shape was uniform and they were flattened, mostly well spread on the sample surface (Fig. 3) [5].

The results showed that both types of hybrid non-wovens

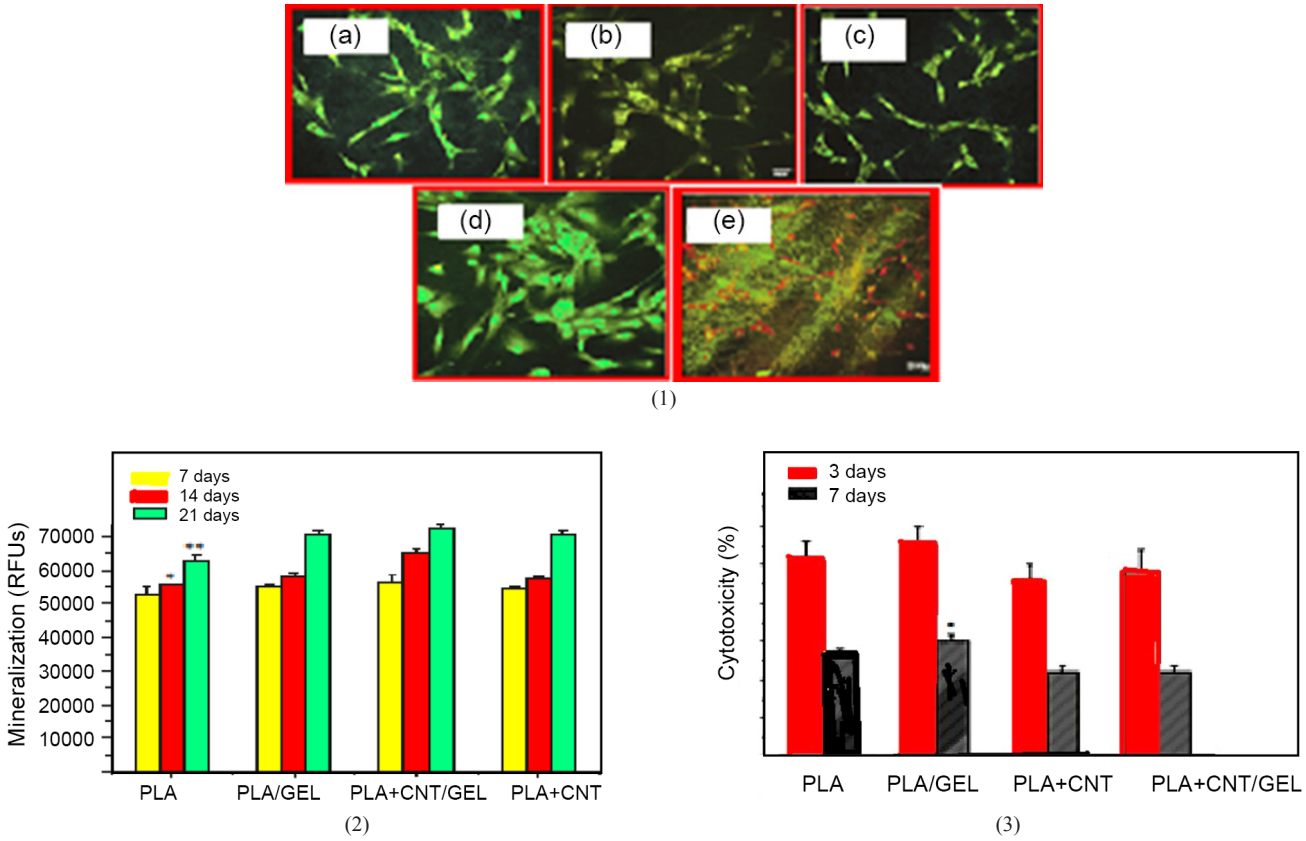
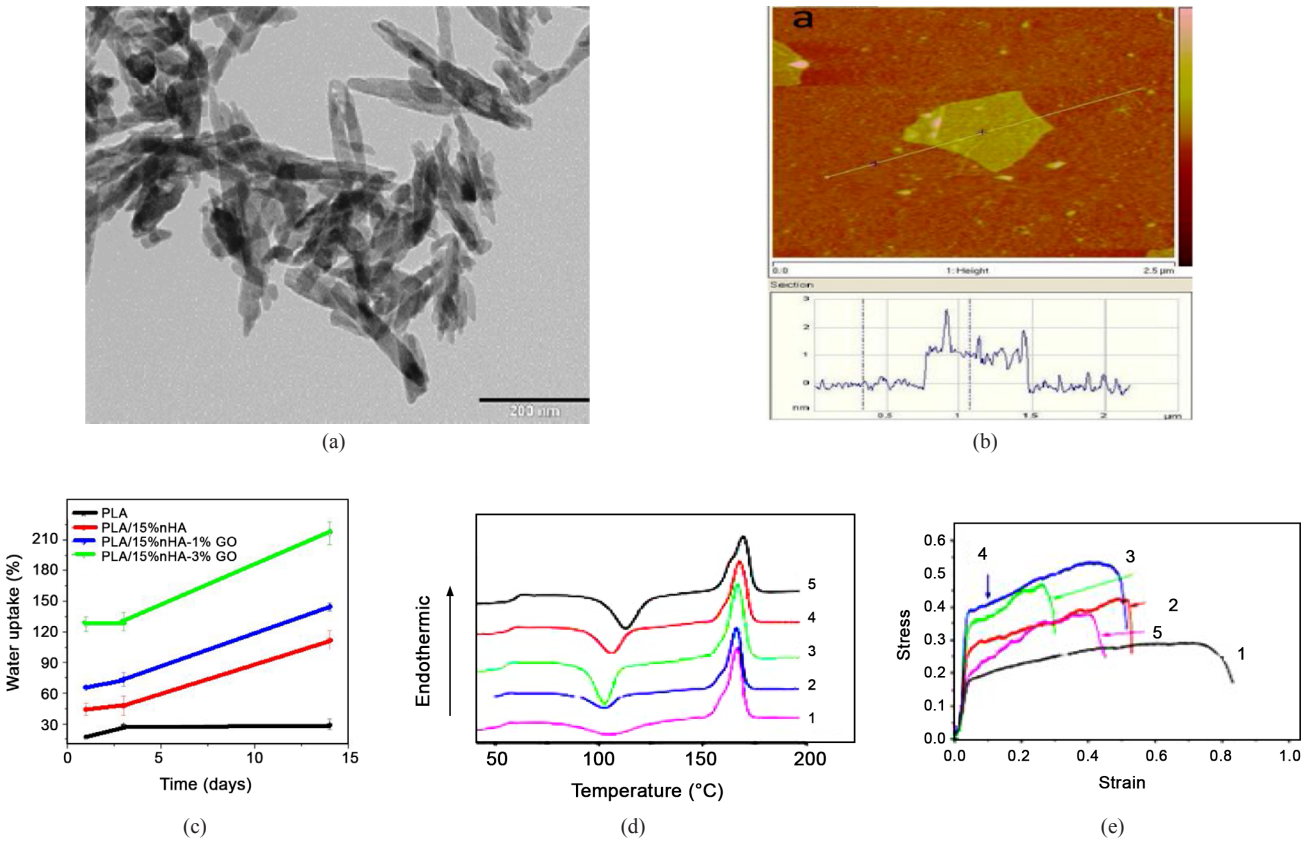



Fig. 3. (1) Morphology (fluorescence microscope) of NHOst cultured on scaffolds after 3 days: (a) PLA (control), (b) PLA/GEL, (c) PLA +CNT/GEL, (d) PLA + CNT, and (e) morphology of NHOst inside PLA/GEL scaffold after 7 days of culture; (2) mineralization progress of NHOst cultured in contact with fibrous scaffolds; *statistically significant (PLA) versus (PLA/GEL) and (PLA + CNT/GEL) on day 14 ($p < 0.05$); **statistically significant (PLA) versus (PLA/GEL) and (PLA + CNT) on day 21 ($p < 0.05$), (3) cytotoxicity for NHOst cultured in contact with fibrous scaffolds; *statistically significant (PLA/GEL) versus (PLA + CNT/GEL) on day 7, ($p < 0.05$) [5].

were not cytotoxic and displayed better osteoinductivity when compared with scaffolds produced from pure PLA and the CNT-contained PLA nanofibres also improved mechanical features of hybrid samples as a bone tissue engineering scaffolds [5].

Liu *et al.* [57] in 2016 studied PLA nanocomposite fiber mats with grapheneoxide (GO) (for developing the mechanical properties of PLA) and nanohydroxyapatite (nHA) (because of its chemical likeness to the inorganic constituent of bone [97,98]). nHA content of 15–18 wt% is required to hold the adhesion and growth of osteoblasts on the scaffold surfaces. Tensile assessment results (Fig. 4) specified that the scaffolds with 15 wt% nHA and 1 wt% GO fillers showed higher tensile strength among the samples studied [57].

In addition, nHA and GO nanofillers increased the water uptake of PLA. So, the scaffolds with 15 wt% nHA and 1 wt% GO having a high tensile strength and modulus, in addition to admirable cell proliferation, are suitable for bone tissue engineering [57]. Nanofibrous tubular PLLA scaffolds were manufactured by centrifugation combined with thermally induced phase separation method utilizing PLLA/1, 4-dioxane/water ternary system, at input voltage of 4.00 V and phase separation temperature of −15 °C [43]. Adding sucrose resulted in the making of porous scaffolds with interconnected pores. Input voltage was the central aspect to make tubular scaffolds that mimicked the structural characteristics of the biological organizations. The surface of nanofibrous tubular scaffold supplied a high-quality environment for attachment and proliferation of MC3T3-E1 (Mouse Osteocalcin) cells, showing considerable potential for bone tissue regeneration [43]. Weili Shao *et al.* [99] studied on producing a biomimetic scaffold comprising of multilayer nano-fiber fabrics (MLNFFs) of PLA and tussah silk fibroin (TSF) that mimic the architecture of the Col fibril matrix in natural lamellar bone. The MLNFF scaffolds were constructed by textile technology (by means of weaving). In particular, woven scaffolds based on a 9:1 mixture of PLA and TSF could be spun constantly into nano-fiber yarns with uniform diameter distribution and perfect mechanical features with a Young's modulus of 417.65 MPa and a tensile strength of 180.36 MPa. In vitro tests indicated that the woven scaffold supports MSC (mouse mesenchymal stem cells)




(a) (b) (c) (d) (e)

Fig. 4. (a) TEM image of nanohydroxyapatite rod (nHA), (b) AFM image of GO with height profile, (c) water absorption behavior of PLA and PLA-based composite nanofiber mats, (d) second heating curves of electrospun, and (e) tensile stress/strain curves of electrospun: (1) PLA, (2) PLA/15%nHA, (3) PLA/15%nHA-1%GO, (4) PLA/15%nHA-2%GO, and (5) PLA/15%nHA-3%GO fibrous mats.

adhesion and proliferation, also develops osteogenesis and mineralization. Mineral deposition with osteoblasts growing on PLA/TSF scaffolds was observed by SEM after 14 days and abundant deposits were noticeably monitored on the surface of cells on PLA/TSF scaffolds. Deposits were made up from Ca and elemental P, as determined by energy dispersive spectrometry. The Ca/P ratio of deposited minerals was 1.58±0.09, and was close to its ratio in pure HA (1.67) [96].

*In vitro* tests (Fig. 5) indicated that the scaffolds notably improved the formation of new bone with characteristic, organization and morphology in damaged femoral condyles through 12 weeks in rabbit femur and the scaffold itself had degraded. The PLA/TSF scaffold is a encouraging approach for bone tissue engineering [99]. The mechanical properties of PLA can be enhanced by the inclusion of calcium phosphate bioceramics like HA to generate composite scaffolds for bone tissue regeneration [27,96,100]. On the other hand, polycaprolactone (PCL) is a biodegradable polyester that is degraded through hydrolysis of ester linkages in the human body and can be used as a substrate for developing the differentiation and proliferation of cells [101,102]. So that PLLA/PCL/HA nanofibrouse scaffolds were studied to mimic the native bone ECM. MC3T3-E1 cells (Mouse Osteocalcin) were cultured on the composite scaffolds. The level of MC3T3-E1 differentiation exhibited higher value with HA containing (PLLA/PCL/HA) than that ones without (PLLA/PCL) and PLLA/PCL/HA nanofibrous scaffold should be an important candidate for proliferation, differentiation and mineralization of osteoblasts [38]. Abdalla *et al.* [103] fabricated PLA nanofiber scaffolds coated with poly(vinyl alcohol) (PVA) with in situ deposition of PVA onto electrospun nanofibers by means of a hydrothermal approach for bone tissue engineering. Addition of hydrophilic elastic polymer onto the surface of the nanofibers scaffold might facilitate to focus on the disadvantages of PLA material (low hydrophilicity and poor ductility). The hydrophilicity of PLA nanofibers scaffold coated with PVA increased significantly (36.11±1.5°) in comparison with that of pure PLA (119.7±1.5°) scaffold. The cytocompatibility outcomes explained that human cells induced more favorable attachment and proliferation on PLA/PVA composite scaffold than that of PLA. Therefore, PVA coating led to an improvement in bone cell attachment

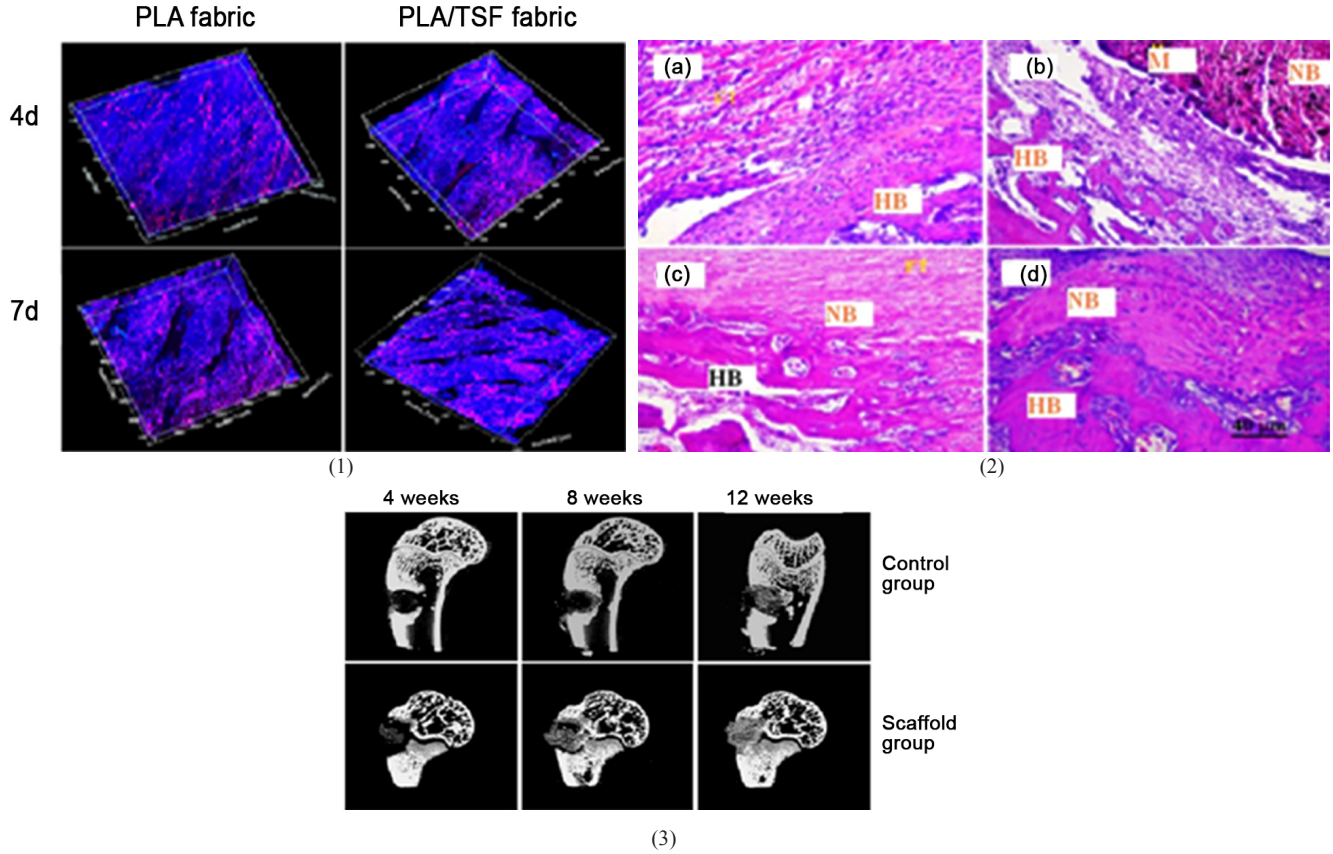



Fig. 5. (1) Confocal fluorescence microscopy of mesenchymal stem cells growing on different substrates for 4 and 7 days, (2) hematoxylin and eosin staining of repaired calvarias in the femur of: (a, c) control animals and (b, d) animals implanted with MLNFFs. Samples were analyzed 10 and 20 weeks after implantation, (3) representative micro-CT images of damaged femoral condyles in rabbits, taken 4, 8, and 12 weeks with or without a PLA/TSF scaffold [99].

and proliferation [100]. Plasma treatment, UV/Ozone technology and alkaline hydrolysis are processes [70,104-106] that can be applied as potential way to alter surface characteristics and the biocompatibility of the scaffolds [104,107-109]. Plasma treatment was used by Sotoudeh *et al.* [110] to add new groups (omentum) on PLA nanofibre scaffolds. The scaffolds were performed by pure oxygen plasma treatment. Omentum involves growth factors like vascular endothelial growth factor (VEGF). It was proved that VEGF activity is necessary for suitable callus architecture and mineralization in return for bone injury [111]. The histological evaluations (Fig. 6) at two, four and six weeks after the implantation demonstrated that PLLA with omentum established appreciably in comparison with the other scaffolds that proposes PLLA with omentum perform as an osteoconductive scaffold [110].

As stated before, bone tissue is a composite of nHA crystals which immersed in a Col and nonCol protein matrix [16]. So that PLA/Col/HA nanocomposite fibers were optimized for osteoconductive bone scaffolds. Polymer coatings generated by electrospinning are regarded as very encouraging bone interfaces owing to the ultrathin-scaled dimensions of their physical construction. The scaffolds were effectively sterilized for the first time by gamma radiation without salient losses in cell-seeding capacity [112]. Lee *et al.* [113] in 2011 showed that PLLA electrospun nanofiber sheets mimic the structure of ECM. They produced electrospun PLLA nano-fibers that were applied with an amino group containing base to produce polymeric nanocylinders as nanofibrous structures for macroporous gelatin scaffolds. They stated that the gelatin/PLLA nano-cylinder composite is a favorable way to generate 3D nanofibrous scaffolds that enhances cell adhesion and proliferation for tissue engineering. Confocal laser scanning microscopy monitoring displayed spreading and flattening cell morphology after culturing NIH3T3 cells on scaffolds in 10 h [109].

Schofer *et al.* [114] developed a methodology applied for growth and osteogenic differentiation of hMSC on electrospun PLLA nano-fibers. The BMP (bone morphogenetic proteins) family is known to improve bone regeneration by increasing a high level of osteoblast activity. They characterized that BMP-2 can be incorporated in a bioactive form using organic solvents without losing its bioactivity into PLLA nano-fibers via electrospinning. When hMSC was seeded and cultured over a time of 22 days on BMP PLLA nanofibers under growth conditions, the primary expression of genes associated with osteoblast lineage was extensively increased by comparison with the gene expression of cells cultured on pure PLLA fibers [114]. A new approach developed by Sui Gang *et al.* [42] whereby the PLLA/HA hybrid membranes were produced with electrospinning of the PLLA/HA dispersion for utilization in bone tissue regeneration. The osteoblast cell (MG-63) was cultured in PLLA/HA hybrid membrane extract holding medium. HA nanoparticles were not only dispersed in the PLLA but as well reacted with the functional group of PLLA, affecting strong surface bonding and high tensile strength of hybrid membrane. The cell adhesion and growth on the PLLA/HA hybrid membrane were significantly higher than those on the pure PLLA membrane [42].

Kim *et al.* [66] developed a new process whereby the bioceramic hydroxyapatite (HA) was kept in suspension in PLA. The approach was to introduce a surfactant hydroxysteric acid (HSA) between the hydrophilic HA

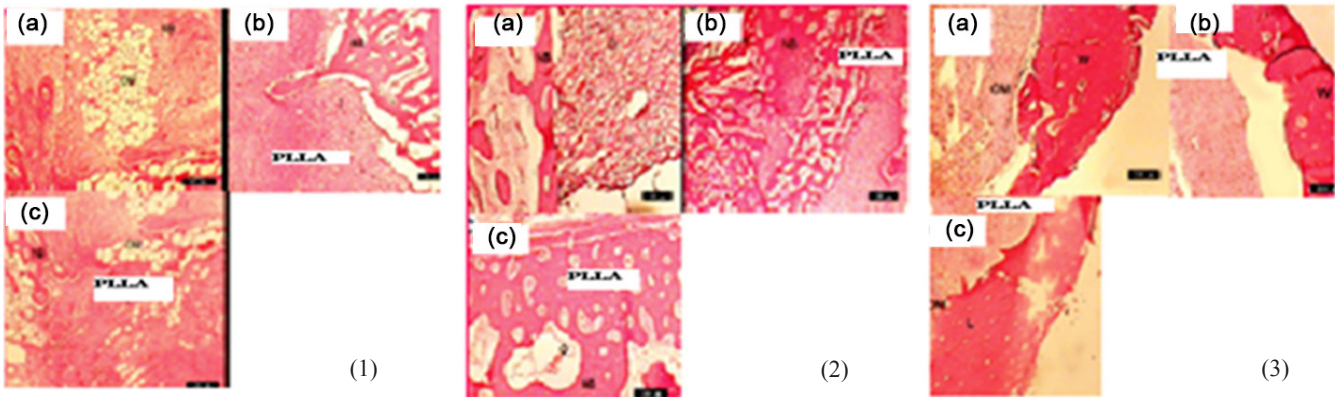



Fig. 6. (1): (a) After two weeks, omentum graft and immature new bone configuration is obvious, (b) ostebelastic rim and direct bone contact with biomaterial and new bone formation is clear, (c) new bone formation is apparent; (2): (a) after four weeks, omentum graft and bone formation is obvious, (b) PLLA and bone formation is apparent, (c) bone formation is evident; (3): (a) after 6 weeks, omentum graft and bone formation and direct contact of bone with graft material is noticeable, (b) PLLA and bone formation is observable, (c) bone formation is obvious [110].

powder and the hydrophobic chloroform-dissolved PLA. The HA nanopowder was dispersed successfully in HSA and mixed equally with PLA. Nanocomposite fibers were generated successfully. Cellular assays illustrated excellent cell attachment and proliferation and also enhanced expression of ALP at 7 days of culturing. So that the HA–PLA biological nano-composite fiber is thought as an encouraging material for bone tissue engineering applications, mainly as 3D substrates for bone growth [66]. Since bone consisted of Col and HA, the Col fibrils are responsible for the toughness and visco-elasticity. The challenge in bone tissue engineering is to expand such composite scaffolds, possesing a balance between biological and biomechanical features. Thus Jonathan B. Chiu *et al.* [115] examined the influence of incorporating very low concentrations (<1 wt%) of Col molecoles within electrospun PLLA on cellular behavior (cellular attachment, viability, proliferation, migration, and differentiation) of constructed scaffolds for bone regeneration. On the basis of the original outcomes, they chose to utilize the 0.83 wt% collagen/PLLA scaffold for more tests. Cells' attachment on 0.83% collagen/PLLA scaffolds after 1, 21, and 42 days are displayed in Fig. 7. Researchers examined cell (MC3T3-E1, mouse osteocalcin) attachment on a forementioned scaffolds. After 3 weeks of culturing cells in medium with differentiation-inducing supplements and staining via von Kossa, nodules indication of calcification were obvious in all 3 groups— tissue culture plate control, PLLA scaffold, and 0.83 wt% collagen/PLLA scaffold.

The results express that the inclusion of Col type I in even low amounts (<1 wt%) within electrospun PLLA scaffolds seem to enhance both cell attachment and migration within the scaffold. Cells could proliferate, penetrate, and differentiate at all concentrations of Col/PLLA mixtures.

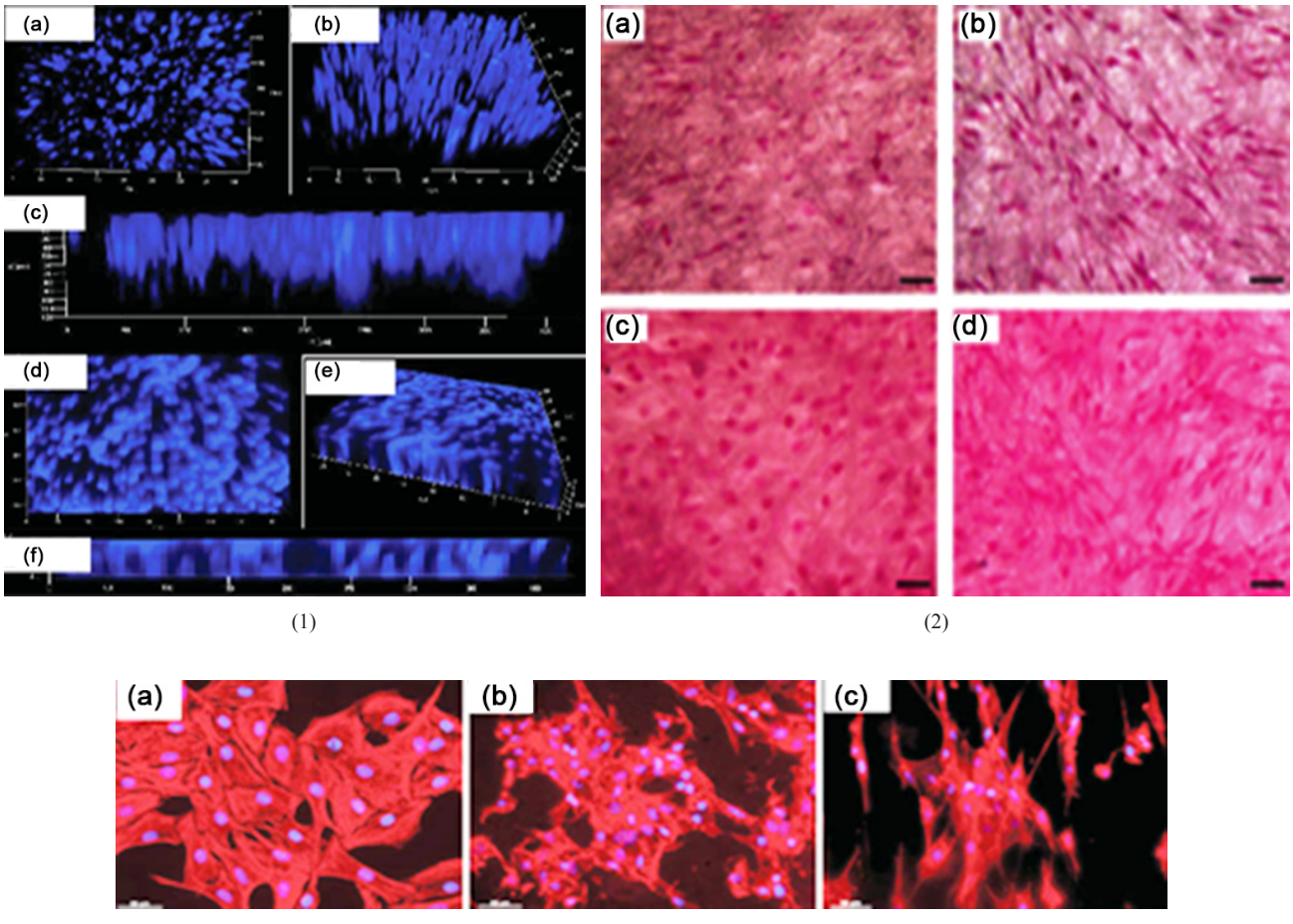



Fig. 7. (1) Three-dimensional visualization of cell migration after 1 week within: (a-c) 0.83% collagen/PLLA and (d–f) PLLA. Electrospun scaffolds in three perspectives: (a, d) top–down, (b, e) diagonal, and (c, f) cross-sectional. After DAPI staining, samples were imaged utilizing the Zeiss Apotome microscope technique; (2) cell attachment on: (a, c) PLLA and (b, d) 0.83% collagen/PLLA scaffolds at: (a, b) 21 days and (c, d) 42 days; (3) cytoskeletal (actin) staining on a: (a) glass coverslip, (b) PLLA scaffold, and (c) 0.83% collagen/PLLA scaffold after 48 h. The well-spread morphology of cells on the glass coverslip (a) are indicative of the flat substrate to which the cells have attached [115].

So, Col molecules could provide as a catalyst in creating a practical double system for implanting cell-scaffolds into critical defects in bone [115]. Cavo *et al.* [15] identified an optimal geometrical pattern of PLA/Col scaffold for bone tissue engineering. Human articular cells were utilized to investigate biocompatibility on scaffolds to examine the the oretical model predictions. The outcomes exhibited a nice agreement between the theoretical model calculations and the experimental information [15].

Chen *et al.* [116] utilized a phase separation technique to produce porous PLLA nanofibrous scaffolds with highly controlled inter-connected sphere-shaped macropores structures. The experimental results revealed that the compressive modulus was enhanced with PLLA concentration. Protein adsorption in those scaffolds was four times higher than that in non-fibrous scaffolds. Also, the attachment of MC3T3-E1 (mouse osteocalcin) cells in serum containing media was 70% more on nano fibrous scaffolds than on non-fibrous-scaffolds. So that, the nanofibres with an interconnected macroporous

TABLE I
PROLIFERATION OF BONE CELLS ON DIFFERENT PLA NANOFIBROUSE SCAFFOLDS

| Type of scaffold | Unit of cell proliferation | Type of cells | Cell proliferation (according to number of the days) | | | | | | | | | | Reference number |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 1 | 2 | 3 | 5 | 7 | 10 | 12 | 14 | 15 | 20 | |
| PLA | Absorbance, 570 nm (MTS Assay) | MSCs | 0. 4 | - | - | - | 1. 4 | - | - | - | - | - | 99 |
| PLA/TSF | Absorbance, 570 nm (MTS Assay) | MSCs | 0. 47 | - | - | | 1. 7 | - | - | - | - | - | 99 |
| PLA | Absorbance, 570 nm | hMSCs | 0. 2 | - | - | - | 0. 67 | - | - | - | - | - | 93 |
| PLA/CH/CS (0.15%) | Absorbance, 570 nm | hMSCs | 0. 3 | - | - | - | 0. 93 | - | - | - | - | - | 93 |
| PLLA | % | hMSC | - | - | - | 9 | - | - | 13 | - | 12 | 10 | 114 |
| PLLA/BMP-2 | % | hMSC | - | - | - | 12 | - | - | 20 | - | 15 | 8 | 114 |
| PLA | Absorbance, 595 nm (MTT Assay) | MG63 | - | 0. 15 | - | - | 0. 25 | - | - | - | - | - | 65 |
| PLA/HA | Absorbance, 595 nm (MTT Assay) | MG63 | - | 0. 1 | | - | 0. 25 | - | - | - | | - | 65 |
| PLLA | Absorbance, 490 nm (MTT Assay) | MC3T3-E1 | 0. 13 | - | - | 0. 15 | - | - | 0.37 | - | - | - | 115 |
| PLLA/ Col (0.83% ) | Absorbance, 490 nm (MTT Assay) | MC3T3-E1 | 0. 11 | - | - | 0. 12 | - | - | 0.34 | - | - | - | 115 |
| PLA | Cell number | NHOst | - | - | 5500 | - | 10000 | - | - | - | - | - | 5 |
| PLA/GEL | Cell number | NHOst | - | - | 5000 | - | 8300 | - | - | - | - | - | 5 |
| PLA/CNT | Cell number | NHOst | - | - | 5000 | - | 9000 | - | - | - | - | | 5 |
| PLA/CNT/GEL | Cell number | NOst | | | 5400 | - | 9200 | - | - | - | - | - | 5 |
| PLA | Absorbance, 570 nm (MTT Assay) | Saos-2 cel | - | - | 0. 15 | - | - | 0. 57 | - | - | - | - | 57 |
| PLA/15%nHA/ 1%GO | Absorbance, 570 nm (MTT Assay) | Saos-2 cel | - | - | 0. 13 | - | - | 0. 63 | - | - | - | - | 57 |
| PLLA | Cell number | mBMSCs | - | - | 2500 | - | 8000 | - | - | 20000 | - | - | 91 |
| ECM/PLLA | Cell number | mBMSCs | - | - | 2000 | - | 10000 | - | - | 35000 | - | - | 89 |

TABLE II
ALP ACTIVITY OF DIFFERENT PLA NANFIBROUSE SCAFFOLDS

| Type of scaffold | Unit of ALP | ALP activity (according to number of the days) | | | | | | | Reference number |
|---|---|---|---|---|---|---|---|---|---|
| | | 3 | 4 | 7 | 10 | 14 | 21 | 22 | |
| PLA | OD405 | - | -- | 0. 2 | 0. 4 | 0. 7 | - | - | 99 |
| PLA/TSF | OD405 | - | - | 0. 25 | 0. 65 | 1. 25 | - | - | 99 |
| PLA | Normalaized to actin | - | - | 0. 3 | - | 0. 4 | - | - | 93 |
| PLA/ CH/CS (0.15%) | Normalaized to actin | - | - | 0. 48 | - | 0. 78 | - | - | 93 |
| PLLA | µmol/mg protein | 0.1 | - | 0,5 | - | 2.7 | - | - | 89 |
| ECM/PLLA | µmol/mg protein | 0.5 | - | 1.5 | - | 3.7 | - | - | 89 |
| PLLA | ALP activity/ALP of the control sample (set to 1) | - | 0. 52 | - | 0. 48 | - | - | 1. 48 | 113 |
| PLLA/BMP-2 | ALP activity/ALP of the PLLA sample (set to 1) | - | 4. 6 | - | 1. 9 | - | - | 0. 95 | 113 |
| PLA | µmol/cells, $\times 10^{-4}$ | - | - | 1. 3 | - | | - | - | 65 |
| PLA/HA | µmol/cells, $\times 10^{-4}$ | - | - | 3. 9 | - | - | - | - | 65 |
| PLA | RFUs | -- | - | 21000 | - | 22000 | 24000 | - | 93 |
| PLA/GEL | RFUs | - | - | 20000 | - | 22500 | 35000 | - | 93 |
| PLA / CNT | RFUs | - | - | 20000 | - | 23500 | 33500 | - | 93 |
| PLA / CNT/GEL | RFUs | - | - | 21000 | - | 26000 | 35000 | - | 93 |
| PLA | µ/mg protein | 7 | - | 13 | - | 27 | - | - | 57 |
| PLA/15%nHA | µ/mg protein | 10 | - | 22 | - | 35 | - | - | 57 |
| PLA/15%nHA/1%GO | µ/mg protein | 12 | - | 31 | - | 46 | - | - | 57 |
| PLA/15%nHA/2%GO | µ/mg protein | 7 | - | 13 | - | 37 | - | - | 57 |

structure may supply a better environment for bone cell distribution, adhesion, proliferation, and differention [95]. Table I sums up the bone cellular activity of different nanofibrouse PLA scaffolds and Table II displays the ALP (ALP is an early marker of hard tissue differentiation and it is generally accepted as an increase in osteoblast) activity of various PLA nanofibrouse scaffolds.

Fig. 8. illustrates the bone cells cultured on different PLA nanofibrouse scaffolds for bone tissue engineering and different PLA and PLLA nanofibrouse scaffolds for bone tissue engineering that were reviewed above.

At last, it can be said that PLA biopolymer has attracted the most attention as a bone tissue engineering material. Furthermore, PLA displays several functions in other biomedical areas, including vascular tissue engineering, wound healing, surgical sutures, bone fixation material, controlled release drug delivery, also as screws, pins, or membranes in bone reconstructive surgery [117,118].

## IV. CONCLUSION

Bone defects and injures are the most important medical trouble and PLA nano-fibrous scaffolds can be thought as a capable key for this difficulty and help bone regeneration. PLA biopolymer has captured the most interest amongst the biodegradeble polymers as a tissue engineering material because it is easily processable and degrades and disintegrates into natural metabolites while matching its degradation rate with the healing time of damaged human tissues. Therefore, this paper reviewed the potential of PLA nanofibres to favour bone tissue engineering because of its biological safety and tuneable degradation features. PLA nanofibres were discussed according to their application forms in bone tissue engineering field as multilayer nanofiber fabrics, porous scaffolds, nanofibres containing HA or as porous scaffolds combined with growth factors (BMP family or omentum). Development of more useful PLA scaffolds with the objective of bone regeneration is a challenging subject in the recent days. Highly porous

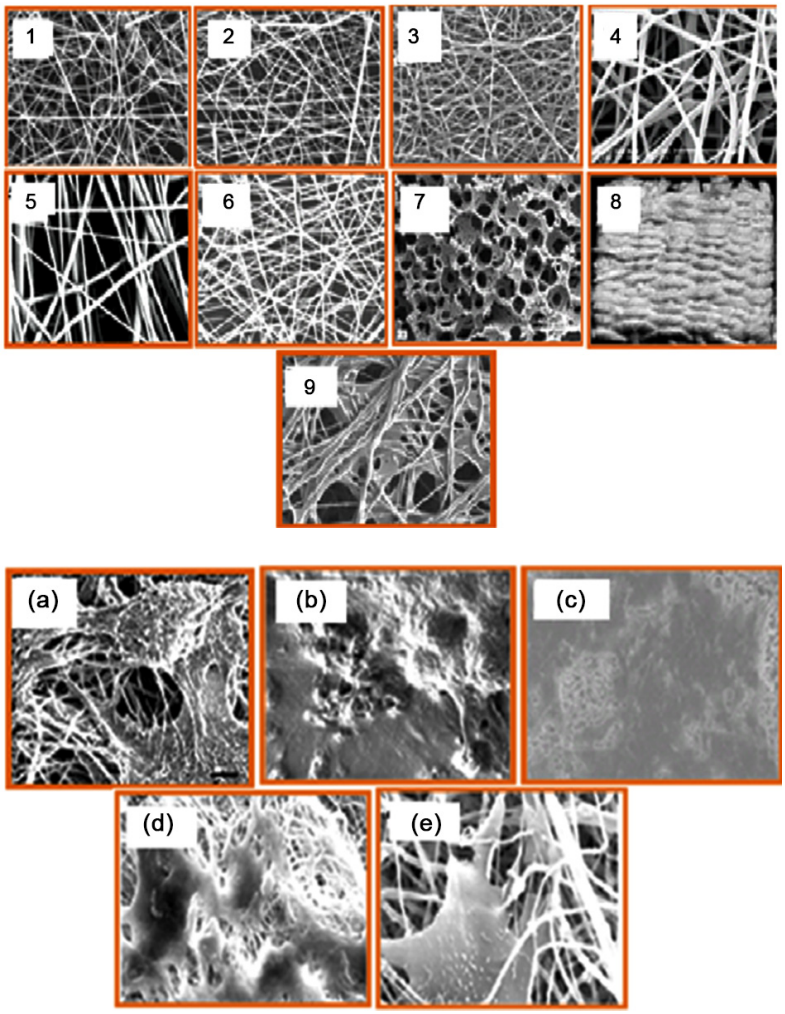



Fig. 8. SEM images of PLA nanofibrous scaffolds used in bone tissue engineering: (1) PLA nanofibrous scaffolds, (2) PLA/CNT nanofibrous scaffolds, (3) PLA/GEL nanofibrous scaffolds, (4) PLLA/Omentum nanofibrous scaffolds, (5) COL/PLLA nanofibrous scaffolds, (6) PLA/CNT/GEL nanofibrous scaffolds, (7) PLLA nanofibrous scaffolds with macropores (sphere size, d=100-250 μm), (8) MLNFF scaffolds fabricated from PLA/TSF nanofibres, (9) ECM/PLLA nanofibrous scaffolds, (a) MG63 cells cultured on HA/PLA scaffolds after 2 days, (b) hMSCs cultured on CS/CH/PLA scaffolds after 1 day, (c) osteoblasts cultured on PLA/nHA scaffolds after 5 days, (d) mesenchymal stem cells cultured on PLA scaffolds after 14 days, and (e) Mbmsc cultured on ECM/PLLA scaffolds after 2 h [57,65,78,89,91,96,113].

three dimentional structure of scaffolds not only implies the better support for cell adhesion but also procures an ideal environment for the migration and proliferation of cells and mimics the architecture of natural fibrillar ECM. Furthermore, the addition of some additives (for instance HA, bone morphogenic protein (BMP-2), omentum and Col type (I) on PLA nanofibrous scaffold could improve the cell adhesion, stem cell differentiation, and tissue formation. However, advances in this field creates more challenges leading to more scientific difficulties which have to be solved and overcome. Finally, the authors propose an alternative strategy "UV/$O_3$ surface functionalization" on PLA scaffolds for improvement of cell adhesion and differentiation. This strategy is expected to increase the success of bone tissue regeneration.

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