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BIODEGRADABLE POLYMERS USED IN TISSUE ENGINEERING

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ABSTRACT

Tissue engineering and regenerative medicines are an exciting research area that aims at regenerative alternatives to harvested tissues for transplantation. Cell, Scaffold and growth factors are the three key materials for tissue engineering. Biomaterials play a pivotal role as scaffolds to provide three dimensional templates and synthetic extracellular matrix environment for tissue regeneration. This paper reviews biodegradable synthetic polymers focusing on their potential in tissue engineering applications. The major classes of polymers are briefly discussed with regard to synthesis, properties and biodegradability, and known degradation modes and products are indicated based on studies reported in the literature. A vast majority of biodegradable polymers studied belongs to the polyester family, which includes polyglycolides and polylactides. Some disadvantages of these polymers in tissue engineering applications are their poor biocompatibility, release of acidic degradation products, poor processability and loss of mechanical properties very early during degradation. Other degradable polymers such as polyorthoesters, polyanhydrides, polyphosphazenes, and polyurethanes are also discussed and their advantages and disadvantages summarised. With advancements in tissue engineering it has become necessary to develop polymers that meet more demanding requirements. Recent work has focused on developing injectable polymer compositions based on poly (propylene fumarate) and poly (anhydrides) to meet these requirements in orthopaedic tissue engineering. Polyurethanes have received recent attention for development of degradable polymers because of their great potential in tailoring polymer structure to achieve mechanical properties and biodegradability to suit a variety of applications.

Key Words: Biodegradable polymers,Degradation, Injectable polymers, Tissue engineering.

I. INTRODUCTION:

Tissue engineering represents an emerging interdisciplinary field that applies the principles of biological, chemical, and engineering sciences towards the goal of tissue regeneration [1].

A distinctive feature of tissue engineering is to regenerate patient's own tissue and organs that are entirely free of poor biocompatibility and low bio functionality as well as severe immune rejection. Cell, scaffold and growth factors are the three key materials for tissue engineering [2]. Cells are often implanted or 'seeded' into an artificial structure capable of supporting three-dimensional tissue formation. These structures are typically called as scaffolds.

Scaffolds usually serve at least one of the following purposes

1. Allow cell attachment and migration

2. Deliver and retain cells and biochemical factors

3. Enable diffusion of vital cell nutrients and expressed products

4. Exert certain mechanical and biological influences to modify the behavior of the cell phase [3].

Prerequisites of scaffolds include

1. Acceptable biocompatibility and toxicity profiles and having ability to support cell growth and proliferation [4].

2. Should have mechanical properties matching those of the tissue at the implantation site or mechanical properties that are sufficient to shield cells from damaging compressive or tensile forces without inhibiting appropriate biomechanical cues [3].

3. The absorption kinetics of scaffold should depend on tissue to be regenerated. For e.g. if scaffold is used for tissue engineering of skeletal system, degradation of scaffold biomaterial should be relatively slow , as it has to maintain the mechanical strength until tissue regeneration is almost completed [2].

4. It should have process ability to form complicated shapes with appropriate porosity. A high porosity and an adequate pore size are necessary to facilitate cell seeding and diffusion throughout the whole structure of both cells and nutrients. An optimum pore size is in the range between 100 and 500 μm[2].

5. Biodegradability is often an essential factor since scaffolds should preferably be absorbed by the surrounding tissues without the necessity of a surgical removal [5].

6. Mimic the native extracellular matrix (ECM), an endogenous substance that surrounds cells, bind them into tissues and provide signals that aid cellular development and morphogenesis.

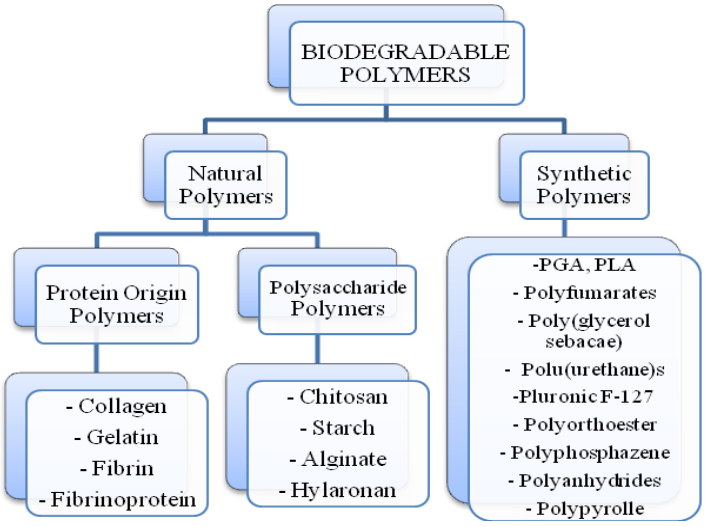
7. Ideally an injectable prepolymers composition should be in liquid/paste form, sterilisable without causing any chemical change, and have the capacity to incorporate biological matrix requirements to be useful in tissue engineering applications. Upon injection the prepolymersmixture should bond to biological surface and cures to a solid and porous structure with appropriate mechanical properties to suit the application. The curing should be with minimal heat generation and the chemical reactions involved in curing should not damage the cells or adjacent tissues [4].

Biodegradable synthetic polymers offer a number of advantages over other materials for developing scaffolds in tissue engineering. The key advantages include the ability to tailor mechanical properties and degradation kinetics to suit various applications. Synthetic polymers are also attractive because they can be fabricated into various shapes with desired pore morphologic features conducive to tissue in-growth. Furthermore, polymers can be designed with chemical functional groups that can induce tissue in-growth.

II. MAJOR CLASSES OF DEGRADABLE POLYMERS

A vast majority of biodegradable polymers studied belong to the polyester family. Among these poly(α-hydroxy acids) such as poly(glycolic acid) (PGA), poly(lactic acid) (PLA), and a range of their copolymers have historically comprised the bulk of published material on biodegradable polyesters and have a long history of use as synthetic biodegradable materials in a number of clinical applications. These polymers have been used as sutures plates and fixtures for fracture fixation devices and scaffolds for cell transplantation.

FLOW CHART FOR THE CLASSIFICATION OF BIODEGRADABLEPOLYMERS



2.1 Poly(Glycolic Acid), Poly(Lactic Acid) and Their Copolymers

Poly(glycolic acid) (PGA) is a rigid thermoplastic material with high crystallinity (46-50%). The glass transition and melting temperatures of PGA are 36 and 225ºC, respectively. Because of high crystallinity, PGA is not soluble in most organic solvents; the exceptions are highly fluorinated organic solvents such as hexafluoro isopropanol. Although common processing techniques such as extrusion, injection and compression moulding can be used to fabricate PGA into various forms, its high sensitivity to hydrolytic degradation requires careful control of processing conditions. Porous scaffolds and foams can also be fabricated from PGA, but the properties and degradation characteristics are affected by the type of processing technique.

Solvent casting, particular leaching method and compression moulding are also used to fabricate PGA based implants.

The preferred method for preparing high molecular weight PGA is ring-opening polymerization of glycolide, the cyclic dimer of glycolic acid, and both solution and melt polymerization methods can be used. The common catalysts used include organo tin, antimony, or zinc. If stannous octoate is used, temperature of approximately 175ºC is required for a period of 2 to 6 hours for polymerization. Although it is possible to synthesize these polymers by acid-catalysed polycondensation of respective acids, the resulting polymers generally have a low molecular weight and often poor mechanical properties.

2.1.1 Biodegradation and Biocompatibility of Polylactides

The degradation of PLA, PGA and PLA/PGA copolymers generally involves random hydrolysisof their ester bonds. PLA degrades to form lactic acidwhich is normally present in the body. This acid then enterstricarboxylic acid cycle and is excreted as water andcarbon dioxide. No significant amounts of accumulationof degradation products of PLA have been reported inany of the vital organs. Carbon13 labelledPLA has demonstrated little radioactivity in faces or urineindicating that most of the degradation

products are releasedthrough respiration. It is also reported that in additionto hydrolysis PGA is also broken down by certainenzymes, especially those with esterase activity. Glycolic acid also can be excreted byurine.The rate of degradation however is determined by factors such as configurational structure, copolymer ratio, crystallinity, molecular weight, morphology, stresses, and amount of residual monomer, porosity and site of implantation.

III. BIOCOMPATIBILITY

Biocompatibility of a material refers to "the ability of a material to perform with an appropriate host response in a specific situation" [40]. It involves not only the material used, but also the surrounding cells/tissue. The interaction of biomaterials and cells is very complex, and only partially understood [41]. To understand the possibilities to orchestrate the biomaterial-cell reactions, a elucidation of their interactions is needed.

For the allowance of initially cell-free polymers to elicit infiltration of cells, this interaction is of pivotal importance. Under physiological conditions, cells will, amongst others, bind to the surrounding extracellular matrix via ligands. Many proteins interact with cells and thereby evoke a myriad of responses [42]. Since the recognition of biomaterials by a cell is typically mediated by proteins [19, 43-46], preadsorption of specific proteins (or small peptides such as Arg-Gly-Asp; RGD) has been investigated to improve cellular response [43, 47, 48]. In general, enhancing the biocompatibility of a biomaterial can be achieved by altering the surface characteristics of the substrate, which in turn can lead to enhancing or reducing protein adsorption [43].

3.1 Biofunctionality

When bony tissues fail due to trauma or disease, additional support is required to take over their mechanical function. For example, in spinal diseases causing degeneration, instability and/or severe deformations, spinal fusion of the segments may be needed. Devices used for this purpose should not only maintain or restore the spinal anatomy, but also create the proper mechanical environment for bony fusion. The load bearing device for interbody spinal fusion is the so-called cage, which usually is supplied with a load-transducing filler material. As bones and implants must resist considerable loads, metals and/or alloys are popular load-bearing materials used for cages.

Metals and/or alloys have proven to be successful, although drawbacks do exist. In spinal surgery, amongst others, permanent materials such as metals (and non-resorbable polymers) remain susceptible to long-term complications such as migration [103] wear [61], late foreign body reaction [61,104] and infection [105]. The inflammatory reaction is, in some cases, the result of the inevitable corrosion of alloys in vivo(often referred to as particle disease), and also in the spine [106,107]. In other cases, the aforementioned micro-motion through the spinal motion segment may lead to particle debris [22].

Therefore strategies to minimize implant related problems have been devised such as removal of the implant after fulfilling its purpose in every patient [108], or to selectively remove the implant in symptomatic patients [95], which in return can cause neurovascular injury or refracture [95]. In the USA, retrieval surgeries of the spine were reported in 25-40% of the patients [109-111]. Furthermore, metallic spinal implants are strongly radiopaque on roentgenograms, which is the most widely used follow-up imaging after spinal surgery [112]. This results in an

obscured view and therefore hampered assessment of fusion, since the presence of a bony bridge throughout the spinal implant cannot be seen [112-115]. Not only do metals/alloys interfere with simple x-ray films, they will therefore also interfere with computer axial tomography scanning (CAT) and cause artefacts (scattering) with magnetic resonance imaging (MRI) [100] In contrast, the presence of a bony bridge on a plain roentgenogram in radiolucent spinal implants can be visualized and does correlate with surgical exploration, considered the gold standard [116]. Radiolucent spinal implants are generally made from non-degradable polymers such as polyetheretherketone (PEEK) and will also not interfere with CAT scans or MRI scans. However, in a similar fashion as metallic cages, non-degradable cages will remain susceptible to similar long-term complications. Development of degradable spinal cages will not only result in optimal assessment of spinal fusion during follow-up using x-ray films, CAT scans or MRI scans, but also avert potential long-term complications, resulting in a patient-friendly and cost-effective treatment option.

3.2 Polylactones

Poly(caprolactone) (PCL) is the most widely studied in this family. PCL is a semicrystalline polymer with a glass transition temperature of about –60ºC. The polymer has a low melting temperature (59 to 64ºC) and is compatible with a range of other polymers. PCL degrades at a much lower rate that PLA and is a useful base polymer for developing longterm, implantable drug delivery systems.

Pol(caprolactone) is prepared by the ring-opening polymerization of the cyclic monomer ε-caprolactone. Catalysts such as stannous octoate are used to catalyse the polymerization and low molecular weights alcohols can be used as initiator which also can be used to control the molecular weight of the polymer.

3.3 Biodegradation And Biocompatibility of Polylactones.

The homopolymer has a degradation timeof the order of two to three years. PCLwith an initial average molecular weight of 50,000 takesabout three years for complete degradation in-vitro. The rate of hydrolysis can be alteredby copolymerisation with other lactones, for example acopolymer of caprolactone and Valero lactone degradesmore readily. Copolymers of ε-caprolactone with dl-lactide have been synthesized to yield materials with more rapid degradation rates (e.g., a commercial suture MONOCRYL, Ethicon). PCL is considered a non-toxic and a tissue compatible material.

Blends with other polymers and block copolymers and low molecular weight polyols and macromeres based on caprolactone backbone are a few of the possible strategies to explore this class of polymers for various applications.

3.4 Poly (Propylene Fumarates)

Recently, polyesters based on fumaric acid have received attention in the development of degradable polymers, and the most widely investigated is the copolyester poly(propylene fumarate) (PPF). The degradation of this copolymer leads to fumaric acid, a naturally occurring substance, found in the tri-carboxylic acid cycle (Krebs cycle), and 1,2-propanediol, which is a commonly used diluent in drug formulations. The copolymer also has unsaturated sites in its backbone, which could be used in subsequent cross-linking reactions.

PPF based degradable polymer compositions including injectable biodegradable materials have been reported in the literature. Injectable systems developed based on PPF have the advantage of employing chemical cross-linking overcoming some of the disadvantages in photo cross-linkable systems. Photo-cross-linkable systems have limited applications for treatment of deep crevices in bone. A number of studies have reported on the synthesis, properties andin-vivodegradation characteristics of poly(propylene fumarate). The copolymers degrade to propylene glycol, poly(acrylic acidco-fumaric acid) and fumaric acid. Cross-linking usually occurs with methyl methacrylate or N-vinyl pyrolidone and benzoyl peroxide as the initiator.

A number of methods have been reported to prepare PPF, and each result in different polymer properties. Products with complex structure are obtained due to side reactions involving different modes of addition. In one method diethyl fumarate and propylene glycol with para-toluene sulfonic acid catalyst are reacted at 250ºC. The yield in this process is only 35 %. In another method, propylene glycol and fumaric acid are heated initially at 145ºC and gradually increasing the temperature to 180ºC. Poly(propylene fumarate) diol with molecular weights in the range 500 to 1200 and polydispersity 3 to 4 can be typically prepared by this method. A third method involves preparing the bis-(hydroxylpropyl) fumarate trimmer and propylene bis(hydrogen maleate) trimmer by reacting propylene glycol/ fumaric acid, and maleic anhydride/propylene glycol, respectively. The two trimmers are then reacted at 180ºC to produce PPF. The bis-(hydroxypropyl) fumarate trimmer can also be prepared at ambient temperature by reacting fumaryl chloride and propylene glycol. The purified trimmer is reacted at 160ºC in the presence of trans esterification catalyst antimony trioxide to produce PPF. PPF with molecular weights in the range 750 to 1500 could be prepared by this method.

The polydispersity ranged from 1.7 to 3. It appears that achieving high molecular weight PPF is difficult because of side reactions, particularly due to the presence of the backbone double bond. Accordingly, incorporation of fillers, or further reactions to form cross-linked networks would be required to achieve good mechanical strength. The mechanical properties vary greatly depending on the method of synthesis and the cross-linking agent used. Mechanical properties could be improved by incorporating ceramic materials such as tricalcium phosphate (TCP), calcium carbonate or calcium sulphate.

These composite materials exhibit compressive strengths in the range 2 to 30 MPa. ß-TCP was particularly useful for reinforcement, and compositions without TCP reinforcement disintegrated very early in the implant.

Cross-linking characteristics reported for PPF, N-vinyl pyrolidone (N-VP), benzoyl peroxide, sodium chloride, and TCP indicate that for a range of formulations, the maximum temperature varied within 38 to about 48ºC, compared to 94ºC observed for polymethylmethacrylate (PMMA) cements. The curing times varied between 1 and 121 min, which allows the composites to be tailored to specific applications. The compressive strengths varied between 1 and 12 MPa.

3.5 Biocompatibility And Biodegradation of Ppf.

PPF undergoes bulk degradation and degradation time is dependent on polymer structure as well as other components. PPF degrades by hydrolysis to fumaric acid and propylene glycol. Based on in-vitrostudies, the time required to reach 20% loss in original weight ranged from 84 (PPF/ß-TCP composite) to over 200 days (PPF/CaSO4 composite). ß-TCP in these compositions not only increased mechanical strength, but also acts as a buffer making the pH change minimal during the degradation process.

PPF does not exhibit a deleterious long-term inflammatory response when implanted subcutaneously in rats. A mild inflammatory response was observed initially and a fibrous capsule formed around the implant at 12 weeks.

IV. POLYANHYDRIDES

Polyanhydrides are one of the most extensively studied classes of biodegradable polymers with demonstrated biocompatibility and excellent controlled release characteristics. Polyanhydrides degrades by surface erosion and their main applications are in controlled drug delivery.Polyanhydrides based drug delivery systems have been utilized clinically.

Polyanhydrides are synthesized by dehydration of the diacid or a mixture of diacids by melt polycondensation. The dicarboxylic acid monomers are converted to the mixed anhydride of acetic acid by reflux in excess acetic anhydride.

High molecular weight polymers are prepared by melt-polycondensation of prepolymers in vacuum under nitrogen sweep.

Langer and co-workers have synthesized polyanhydrides for drug delivery applications. Polyanhydrides is used to deliver carmustine, an anticancer drug, to sites in the brain where a tumour has been removed. The degradation products of are non-toxic and have controlled surface erosion degradation mechanism that allows delivery of drugs at a known rate. Polyanhydrides have limited mechanical properties that restrict their use in load–bearing applications such as in orthopaedics. For example poly[1,6-bis(carboxyphenoxy) hexane] has a Young's modulus of 1.3 MPa which is well below the modulus of human bone (40 to 60 MPa). To combine good mechanical properties of polyimides with surface-eroding characteristics of polyanhydrides, poly(anhydrides-co-imides) have been developed, particularly for orthopaedic applications. Examples include poly-[trimellitylimidoglycine-co bis(carboxyphenoxy) hexane], and poly[pyromellitylimidoalanine-co-1,6-bis(carboph-enoxy)-hexane]. These poly(anhydride-co-imides) have significantly improved mechanical properties, particularly compressive strengths. Materials with compressive strengths in the 50 to 60 MPa range have been reported for poly(anhydrides-co-imides) based on succinic acid trimellitylimidoglycine and trimellitylimidoalanine. The degradation of these copolymers occurred via hydrolysis of anhydride bonds, followed by the hydrolysis of imide bonds.

Photo cross-linkable polyanhydrides have also been developed for use in orthopaedic applications, particularly focusing on achieving high mechanical strength. The systems developed are based on dimethacrylated anhydrides.

Dimethacrylatedmacromeres based on sebacic acid and 1,6-bis(p-carboxyphenoxy)hexane. Both ultraviolet (UV) and visible light cure methods have been investigated with these macro monomers. The most effective means of photo polymerization of these macro monomers was found to be 1.0 wt % camphor Quinone and 1.0 wt % ethyl-4-N,N-dimethyl amino benzoate with 150 mW/cm2. Combination of redox type and visible initiation has provided means of achieving efficient curing of thick samples.

Depending on the monomers used, the mechanical properties as well as degradation time can be varied.

Compressive strengths of 30-40 MPa, and tensile strengths of 15-27 MPa, similar to those of cancelleous bone, have been reported.

4.1 Biocompatibility And Biodegradation of Polyanhydrides.

Polyanhydrides are biocompatible, have well-defined degradation characteristics, and have been used clinically in drug delivery systems. Polyanhydrides degrade by hydrolysis of the anhydride linkage. The hydrolytic degradation rates can be altered by simple changes in the polymer backbone structure by choosing the appropriate diacid monomers.

Poly(sebasic acid) degrades quickly (about 54 days in saline), while poly(1,6-bis(-*p*-carboxyphenoxy)hexane degrade much more slowly (estimated 1 year). Accordingly, combinations of different amounts of these monomers would result in polymer with degradation properties custom- designed for a specific application. Minimal inflammatory responses to sebacic acid/1,3- bis(p-carboxyphenoxy) propane (SA/CPP) systems have been reported when implanted subcutaneously in rats up to 28 weeks. Loose vascularized tissue had grown into the implant at 28 weeks, with no evidence of fibrous capsule formation. No data have been reported about polymer sterilizability and heat generation during polymerization. A 12 week study using 2-3 mm diameter full thickness defect in the distal femur of rabbits showed good tolerance of the SA/CPP polymer system and osseous tissue in the outer zone of some implants.

V. CONCLUSIONS

A vast majority of biodegradable polymers studied belong to polyester family and poly(glycolic acid), poly (lactic acid) and their copolymers have historically comprised the bulk of published material. These polymers have a relatively long history of use in a number of clinical applications.

They will continue to play a key role in various forms for medical applications requiring biodegradable polymers. Polyesters offer synthetic chemists many opportunities to design polymers through combination of different monomers to achieve property requirements to suit a variety of applications. Additionally, the development of precursors such as polyols and macro monomersbased on polyesters may find uses in injectable and *insitu*curable polymer formulations. Poly(propylene fumarate) is one example of a recently developed polyester basedinjectable polymer system.

Polyanhydrides is another family of polymers studies extensively with demonstrated biocompatibility and excellent controlled release characteristics. Polyanhydridesdegrade by bulk erosion and their main applications are in controlled drug delivery. Recently photocross-linkable polyanhydrides have been developed for use in orthopaedic applications. Tyrosine-derived polycarbonates,polyorthoesters, polyurethanes and polyphosphazenes have also been investigated to explore their potential as biodegradable polymers.

Review of the literature indicates that relatively few attempts have been made to develop injectable polymer compositions for use in tissue engineering applications. The key challenges in developing such compositions include the choice of appropriate precursors that would degrade to biocompatible and resorbable compounds, the ability to incorporate cells and other components to support cell attachment and proliferation, ability to cure

insitu in the *in-vivo* environment with minimal heat generation, and the ability to control degradation kinetics to suit the intended application.

Polyurethanes offer many advantages in the design of injectable and biodegradable polymer compositions. As a class of polymers, polyurethanes generally have good biocompatibility. They also offer substantial opportunities to tailor polymer structure to achieve a broad range of mechanical properties. By choice of star, dendritic or hyperbranched prepolymers, one can introduce structural variations to tailor degradation kinetics as well as incorporation of appropriate functional groups for improved cell attachment. In the rapidly advancing field of tissue engineering, polyurethanes offer numerous opportunities to develop suitable scaffolds for a variety of applications.

REFERENCES

[1] R.Vasita and D.S. Katti, Nanofiber and their applications in tissue engineering,Int J Nanomedicine, 1,15“30 (2006).

[2] Y. Ikada, Challenges in tissue engineering, J R Soc interface, 3, 589-601(2006).

[3] P.M. Sokolsky, K. Agashi, A. Olaye, K. Shakesheff and A.J. Domb, Polymer carriers for drug delivery in tissue engineering, Adv. Drug Deliv. Rev., 59, 187“206 (2007).

[4] P.A.Gunatillake and R. Adhikari, Biodegradable synthetic polymers for tissue engineering. Eur. Cell Mater.,5, 1-16(2003).

[5] G.u. Haigang, Y. Zhilian, N. Bramasta , W.S. Leong and J.P. Tan, Control of in vitro neural differentiation of mesenchymal stem cells in 3D macroporous, cellulosic hydrogels, Regenerative Medicine, 5, 245-253 (2010).

[6] S.R. Bhattarai, N. Bhattarai, H.K. Yi, et al., Novel biodegradable electrospun membrane: scaffold for tissue engineering, Biomaterials,25, 2595“602(2004).

[7] D.H. Reneker and I. Chun, Nanometer diameter fibers of polymer produced by electrospinning, Nanotechnology, 7, 216“23 (1996).

[8] Y.M. Shin, M.M. Hohman and M.P. Brenner, Electrospinning: a whipping fluid jet generates submicron polymer fibers,ApplPhysLett,78, 1-3(2001).

[9] S.V. Fridrikh, J.H. Yu, M.P. Brenner and G.C. Rutledge, Controlling the fiber diameter during electrospinning, Phys RevLett.,90,144502(2003).

[10] M.M. Hohman,M. Shin and G. Rutledge, Electrospinning and electrically forced jets I. Stability theory, Phys Fluids, 13, 2201“20 (2001).

[11] M.M. Hohman, M. Shin, G. Rutledge and M.P. Brenner, Electrospinning and electrically forced jets. II Applications, PhysFluids,13, 1-16(2001).

[12] J. Doshi and D.H. Reneker, Electrospinning process and application of electrospun fibers, J Electrostat, 35,151“160(1995).

[13] A.L. Yarin , S. Koombhongse and D.H. Reneker, Taylor cone and jetting from liquid droplets in electrospinning of nanofibers, J ApplPhys, 93, 4836“4846 (2001).

[14] J. Zeleny, The electrical discharge from liquid points and a hydrostatic method of measuring the electric intensity at their surface, Phys Rev.,3, 69-91(1914).

[15] D.H. Reneker , A.L. Yarin, H. Fong and S. Koombhangse, Bending instability of electrically charged liquid jets of polymer solutions in electrospinning, J ApplPhys, 87, 4531“4547 (2000).

[16] Y.M. Shin, M.M. Hohman and M.P. Brenner, Experimental characterization of electrospinning: The electrically forced jet and instabilities, Polymer,42, 9955“9567(2001).

[17] A. Frenot and I.S. Chronakis, Polymer nanofibers assembled by electrospinning, CurrOpinColl Inter Sci.,8, 64-75 (2003).

[18] K. Jayaraman , M. Kotakim , Y. Zhang ,X. Mo and S. Ramakrishna, Recent Advances in Polymer Nanofibers, Journal of Nanoscience and Nanotechnology,4, 52-65(2004).

[19] A.G. Mikos, A.J. Thorsen , L.A. Czerwonka,Y. Bao,R. Langer, D.N. Winslow DN, et al., Preparation and Characterisation of Poly (LLacticAcid) Foams, Polymer, 35, 1068-1077(1994).

[20] S.L. Edwards, W. Mitchell, J.B. Matthews, E. Ingham and S.J. Russell, Design of Nonwoven Scaffold Structures for Tissue Engineering Of the Anterior Cruciate Ligament, Autex Research Journal, 4, 86-94(2004).

[21] V.P. Shastri, R. Martin and R. Langer, Microporous polymer foams by hydrocarbon templating in Proceeding of the national academy of sciences ,USA (2000) p 1970-1975.

[22] L. Xiaohua and X.M. Peter, Polymeric Scaffolds for Bone Tissue Engineering, Annals of Biomedical Engineering, 32, 477“ 486(2004)

[23] K. Whang, C.H. Thomas, K.E. Healy and G.A. Nuber, Novel method to fabricate bioabsorbablescaffolds, Polymer, 36, 837-842 (1995).

[24] L.H. Kadiyala, S. Guggino and K.W. Leong, Poly (L-lactic acid) foams with cell seeding and controlled-release capacity, Journal of Biomedical Materials Research Part A, 30,475-484 (1998).

[25] H. Lo, M.S Ponticiello and K.W. Leong, Fabrication of controlled release biodegradable foams by phase separation, Tissue Engineering, 1, 15-28(1995).

[26] C. Schugens, V. Maquet, C. Grandfils, R. Jerome and P. Teyssie, Polylactidemacroporousbiodegradable implants for cell transplantation II, Preparation of polylactide foams for liquid-liquid phase separation, Biomed Mater Res., 30, 449-61(1996).

[27] Y.S Nam and T.G. Park, Porous biodegradable polymeric scaffolds prepared by thermally induced phase separation, J. Biomed. Mater.Res. ,47, 8-17(1999).

[28] Y.S. Nam and T.G. Park, Biodegradable polymeric microcellular foams by modified thermally induced phase separation method, Biomaterials, 20, 1783- 1790(1999).

[29] L.H. Sperling, Introduction to physical polymer science, Macromolecular Chemistry and Physics, 30, 787(2006).

[30] D.J. Mooney, D.F. Baldwin, N.P. Suh, J.P. Vacanti and R. Langer, Novel approach to fabricate porous sponges of poly D,Llactic- co-glycolic acid) without the use of organic solvents, Biomaterials, 17,1417-1422(1996).

[31] A.I. Cooper, Polymer synthesis and processing using supercritical carbon dioxide, J. Mater. Chem, 10, 207-234 (2000).

[32] L.D. Harris, B.S. Kim and D.J. Mooney, Open pore biodegradable matrices formed with gas foaming, J. Biomed. Mater. Res.,42,396- 402(1998).

[33] T.G. Park, New approaches to fabricate highly porous tissue scaffolds, Electronic Journal of Biotechnology,15, 4 (2000).

[34] Y.S. Nam, J.J. Yoon and T.G. Park, A novel fabrication method of macroporous biodegradable polymer scaffolds using gas foaming salt as a porogen additive, Journal of Biomedical Materials Research,53, 1-7(2000).

[35] S.F. Yang, K.F. Leong, Z.H. Du and C.K. Chua, The design of scaffolds for use in tissue engineering Part II Rapid prototyping techniques, Tissue Eng, 8,1-11(2002).

[36] H. Yoshimoto, Y.M. Shin, H. Terai and J.P. Vacanti, A biodegradable nanofiber scaffold by electrospinning and its potential for bone tissue engineering, Biomaterials,24,2011-2082(2003).

[37] H.S. Lo, S.E. Guggino and K.W. Leong, Poly (Lactic acid) foams with cell seeding and controlled-release capacity, J. Biomed. Mater. Res.,30,475-484(1996).

[38] Y.S. Nam and T.G. Park, Porous biodegradable polymeric scaffolds prepared by thermally induced phase separation, J. Biomed. Mater.Res., 47, 8-17(1999).

[39] R. Zhang and P.X. Ma, Processing of polymer scaffolds: Phase separation, in: Methods of Tissue Engineering, (ed) A. Atala andR. Lanza, Academic Press: United states, 715“724(2001).

[40] P.X. Ma, R.Y. Zhang, G.Z. Xiao and R. Franceschi, Engineering new bone tissue in vitro on highly porous poly(alpha-hydroxyl acids)/hydroxyapatite composite scaffolds, J. Biomed. Mater. Res.,54, 284-293(2001).

[41] A.G. Mikos, M.D. Lyman, L.E. Freed and R. Langer, Wetting of poly (L-lactic acid) and poly(DL-lactic-co-glycolic acid) foams for tissue culture, Biomaterials,15, 55-68(1994).

[42] J.A. Neff, K.D. Caldwell and P.A. Tresco, A novel method for surface modification to promote cell attachment to hydrophobic substrates, J. Biomed. Mater.Res., 40, 511-519(1998).

[43] M. Yamamoto, K. Kato and Y. Ikada, Ultrastructure of the interface between cultured osteoblasts and surface-modified polymer substrates, J. Biomed. Mater.Res., 37, 29“36(1997).

[44] R.Y. Zhang and P.X. Ma, Porous poly (L-lactic acid)/apatite composites created by biomimetic process, J. Biomed. Mater.Res.,45, 285“293(1999).

[45] X. Peter, Biomimetic materials for tissue engineering .Advance drug delivery rev, 60, 184“198(2008).

[46] S.P. Massia, M.M. Holecko and G.R. Ehteshami, In vitro assessment of bioactive coatings for neural implant applications, J. Biomed. Mater.Res., **68,** 177“186 (2004).

[47] E. Broderick, S. Infanger, T.M. Turner and D.R. Sumner, Depressed bone mineralization following high dose TGF-beta1 application in an orthopedic implant model, Calcif. Tissue Int., 76, 379“384(2005).

[48] L. McKinney and J.O. Hollinger, A bone regeneration study: transforming growth factor-beta 1 and its delivery, J. Craniofac. Surg,7,36-45(1996).

[49] M.A. Critchlow, Y.S. Bland and D.E. Ashhurst, The effect of exogenous transforming growth factor-beta 2 on healing fractures in the rabbit, Bone, 16, 521“527(1995).

[50] M. Stephanie, Willerth, E. Shelly and E. Sakiyama, Approaches to neural tissue engineering using scaffolds for drug delivery, Adv. Drug Deliv. Rev.,59, 325-338(2007).

[51] G. Schmidmaier, M. Lucke, P. Schwabe, M. Raschke, N.P. Haas and B. Wildemann, Collective review: bioactive implants coated with poly (d,l-lactide) and growth factors IGF-I, TGF-beta1, or BMP-2 for stimulation of fracture healing, J. Long-Term Eff. Med. Implants.,16, 61“69(2006).

[52] J. Street, M. Bao, L. DeGuzman, S. Bunting, F.V. Peale, N. Ferrara , et al., Vascular endothelial growth factor stimulates bone repair by promoting angiogenesis and bone turnover, Proc. Natl. Acad. Sci., 99, 9656“9661(2002).

[53] C. Becker, D.L. Olde, T. Laeufer, B. Brehmer, I. Heschel and G. Jakse, Novel collagen matrices for application in tissue engineering of the urinary tract, International journal of artificial organs, 29, 764-771(2006).

[54] M.R. Urist, Bone: Formation by Autoinduction, Science, 12, 893“899(1965).

[55] J.M. Wozney, V. Rosen, A.J. Celeste, L.M. Mitsock, M.J. Whitters, R.W. Kriz, et al ., Novel regulators of bone formation: molecular clones and activities, Science, 242, 1528“1534(1988).

[56] S. Huang, T. Deng, W. Yijuan, D. Zhihong, H.Lisheng , L. Shouxin, et al., Multifunctional implantable particles for skin tissue regeneration: Preparation, characterization, in vitro and in vivo studies, ActaBiomaterialia, 4 , 1057-1066(2008).

[57] Y. Chunlin, P.J. Hillas, J.A. Buez,M. Nokelainen, J. Balan, J. Tang , et al., The application of recombinant human collagen in tissue engineering, BioDrugs, 18, **103**“119(2004).

[58] C. Danielsson, S. Ruault, D.A. Basset and P. Frey, Modified collagen fleece, a scaffold for transplantation of human bladder smooth muscle cells, Biomaterials,27, 1054“10609(2006).

[59] C.D. Chin, K. Khanna and K. Samuel, Biomed microdevices: A microfabricated porous collagen-based scaffold as prototype for skin substitutes, Biomaterials,10, 456-467(2008).

[60] K.J. Shields, M.J. Beckman and G.L. Bowlin. Mechanichal properties and cellular proliferation of electrospun collagen type II, Tissue eng, 10, 1510-1517(2004).

[61] I.V. Yannas, J.F. Burke, P.L. Gordon, C. Hung and R.H. Rubenstein, Design of an artificial skin. II. Control of chemical composition, J. Biomed.Mater. Res., 14, 107“132(2004).

[62] C. Auxenfans, B.V. Andre, C.A. Lequeux, S. Rose, F. Braye, J. Fradette et al ., Porous matrix and primary-cell culture: A shared concept for skin and cornea tissue engineering, PatholBiol (2008).

[63] T. Aper, A. Schmidt, M. Duchrow and H.P. Bruch, Autologous blood vessels engineered from peripheral blood sample, Eur. J., **33,** 33“39(2007).

[64] 28 H. Patel, M. Bonde, G. Srinivasan64. Y. Tamada, New process to form a silk fibroin porous 3-D structure, Biomacromolecules, 6, 3100“3106,(2005).

[65] C.B. Herbert, G.D. Bittner and J.A. Hubbell, Effects of fibinolysis on neurite growth from dorsal root ganglia cultured in two- and three-dimensional fibrin gels, J. Comp. Neurol., **365,** 380“391(1996).

[66] S.E Sakiyama, J.C. Schense and J.A. Hubbell, Incorporation of heparin-binding peptides into fibrin gels enhances neuriteextension: an example of designer matrices in tissue engineering, FASEB Jornal,13, 2214“2224(1999).

[67] T. Ahmed, A.E. Tamer, E.V. Dare and M. Hincke, Fibrin: A Versatile Scaffolds for Tissue Engineering Application, Tissue engineering part B: Review, 14, 199-215(2008).

[68] S. Sofia, M.B. MacCarthy, G. Gronowiz and D.L. Kaplan. Functionalized silk based biomaterials, J. Biomed. Mater. Res, 54 ,139-148(2001).

[69] P.B. Malafaya, G.A. Silva, R.L. Reis, Natural“origin polymers as carriers and scaffolds for biomolecules and cell delivery intissue engineering applications, Adv. Drug Deliv.Rev., 59, 207-233 (2007).

[70] M. Li, M.J. Mondrinos, M.R. Gandhi, F.K. Ko, A.S. Weissa, P.I. Lelkes, et al., Electrospun protein fibers as matrices for tissue engineering, Biomaterials,26,5999“6008(2005).

[71] S. Huang, T. Deng, W. Yijuan, D. Zhihong, H. Lisheng ,L.Shouxin L, et al., Multifunctional implantable particles for skin tissue regeneration: Preparation, characterization, in vitro and in vivo studies, ActaBiometer, 4,1057-1066(2008).

[72] S.A. Sell, M.G. McClure, C.P. Barnes, D.C. knapp, B.H. Walpoth, D.G. Simpson, et al., Electrospunpolydioxanone“elastinblends: potential for bioresorbable vascular grafts, Biomed. Mater,1,72-80(2006).

[73] Y. Xiaojun, P.D. George and V.B. Ravi, A Laminin and Nerve Growth Factor-Laden Three-Dimensional Scaffold for Enhanced Neurite Extension, Tissue Engineering,5, 291-304(1999).

[74] M. Alaminos, I.M. Jose, D. Serrano, S. Medialdea, I. Carreras, A. Campos , et al., Construction of a Complete Rabbit Cornea Substitute Using a Fibrin-Agarose Scaffold, Investigative Ophthalmology and Visual Science, 47,3311-3331(2006).

[75] D.B. Saris , N. Mukherjee, L.J. Berglund, F.M. Schultz, K.N. An, S.W. O’Driscoll, Dynamic pressure transmission through agarose gels, Tissue Eng., 6 ,531-537(2000).

[76] S. Sakai, I. Hashimoto and K. Kawakami, Synthesis of an Agarose-Gelatin Conjugate for Use as a Tissue Engineering Scaffold, Journal of Bioscience and Bioengineering,1309,22-26(2007).

[77] S.P. Massia, M.M. Holecko and G.R. Ehteshami, In vitro assessment of bioactive coatings for neural implant applications, J Biomed Mater Res A, 68, 177“186(2003).

[78] C.L. Klaver and M.C. Caplan, Bioactive surface for neural electrodes: decreasing astrocyte proliferation via transforming growth factor beta-1, J Biomed Mater Res A., 81, 1011-1016(2007).

[79] S.G. Levesque and M.S. Shoichet, Synthesis of cell-adhesive dextran hydrogels and macroporous scaffolds, Biomaterials,27, 5277“85(2006).

[80] E. Khor and L.Y. Lim, Implantable applications of chitin and chitosan,Biomaterials, 24, 2339“2349(2003).

[81] Y. Okamoto, R. Yano, K. Miyatake, I. Tomohiro, Y. Shigemasa and S. Minami, Effects of chitin and chitosan on blood coagulation, Carbohyd. Polym., 53, 337“342(2003).

[82] D.K. Kweon, S.B. Song and X.Y. Park, Preparation of water-soluble chitosan/heparin complex and its application as wound healing accelerator, Biomaterials, 24, 1595“1601(2003).

[83] H. Ueno, T. Mori and T. Fujinaga, Topical formulations and wound healing applications of chitosan, Adv. Drug Deliv. Rev.,52,105- 115(2001).

[84] C. Alemdaroglu, Z. Degim, N. Celebi, F. Zor, S. Ozturk and D.Erdogan, An investigation on burn wound healing in rats with chitosan gel formulation containing epidermal growth factor, Burns,32,319-37(2006).

[85] P.M. George, A.V. Lyckman, D.A. LaVan, A. Hegde, Y. Leung, R. Avasare, C. Testa , et al., Fabrication and biocompatibility of polypyrrole implants suitable for neural prosthetics, Biomaterials,26, 3511“3519(2005).

[86] L.L. Hench, Artificial organs and tissue engineering, in: Biomaterials,(ed) (ed) L.L. hench andJ.R.Jones, Woodhead Publishing Limited: England 36.8 - 36.9.(2005).

[87] S. Bhattacharyya, L.S. Nair, A. Singh, N.R. Krogman, J. Bender and Y.E. Greish, et al, Development of Biodegradable Polyphosphazene- Nanohydroxyapatite Composite Nanofibers Via Electrospinning,in proceeding of the Materials Research Society, 2005, p- 91-96.

[88] M. Martina and D.W. Hutmacher, Biodegradable polymers applied in tissue engineering research: A review, Polym. Int.,56, 145“ 157(2007).

[89] M.A. Ambrosio, J.S. Sahota, C. Runge, S.M. Kurtz, S. Lakshmi, H.R. Allcock and C.T. Laurencin, Novel phosphazene“ hydroxyapatite composites as biomaterials, IEEE Eng. Med. Biol. Mag22,19-26(2003).

[90] C.T. Laurencin and L.S. Nair, Polyphosphazenes nanofiber for biomedical applications: preliminary studies, in: Nonoengineerednanofibers materials, (ed) I. Selcuk, G. Gogusti, V.K. Vladimir, H.R. Allcock and C.T. Laurencin, Kluwer academic publisher: Turkey , p- 281-298(2003).

[91] D. Joseph, Tissue engineering and artificial organ. Technology & Engineering, 1304(2006).

[92] K.E. Uhrich, inventor, Rutgers, Assignee, Polyanhydrides with therapeutically useful degradation products, United States Patent 20070213500.2007 September 13.

[93] C.J. Campo, T. Anastasiou and K.E. Uhrich, Polyanhydrides: The effects of ring substitution changes on polymer properties, Polymer bulletin,42,61-68(1999).

[94] J. Gao, P.M. Crapo and Y.D. Wang, Macroporous elastomeric scaffolds with extensive micropores for soft tissue engineering, Tissue Eng,12, 917“925 (2006) .

[95] O. Bostman, E. Hirvensalo, S. Vainionpaa, A. Makela, K. Vihtonen, P. Tormala, et al., Ankle fractures treated using biodegradable internal-fixation, Clin. Orthop.Relat.Res., 238, 195“203(1989).

[96] D.C. Chen, R. Jeffrey, V.G. Agopian, V.D. Hoagland, J.D. Woolman, P. Shenget,et al., Comparison of Polyester Scaffolds for Bioengineered Intestinal Mucosa, Cells Tissues Organs, 184, 154-165(2006).

[97] J.M. Moran, David, B.S. Pazzano and L.J. Bonassar, Characterization of PolylacticAcid“Polyglycolic Acid Composites for Cartilage Tissue Engineering, Tissue Engineering, 9, 63-70(2003).

[98] S. Rizk, D.P. Martin, K. Ho, A. Ganatra and S.F. Williams, inventors, Tepha, Inc. ,assignee, Toughened Polylactic Acid Polymers and Copolymers Document, United States Patent 20070182041,2007 November 14.

[99] Z. Junchuan, W. Linbo, J. Dianying and D. Jiandong, Fabrication of Three-Dimensional Porous Scaffolds of Complicated Shape for Tissue Engineering. I, Compression Molding Based on Flexible"Rigid Combined Mold.Tissueengineerin, 11, 1105-1114 (2005).

[100] M.S. Cortizo, S.M. Molinuevo, A.M and Cortizo, Biocompatibility and biodegradation of polyester and polyfumaratebasedscaffoldsfor bone tissue engineering, Journal of tissue engineering and regenerative medicines, 2, 33-42(2008).

[101] Z. Junchuan, W. Linbo, J. Dianying and D. Jiandong, A comparative study of porous scaffolds with cubic and spherical macropores, Polymer, 46, 4979"4985(2005).

[102] A.R. Webb, J. Yang and G.A. Ameer, Biodegradable polyester elastomers in tissue engineering, Expert OpinBiol Ther.,4,801-12(2004).

[103] I. Nagura, H. Fujioka, T. Kokubu, T. Makino, Y. Sumi and M. Kurosaka**,** Repair of osteochondral defects with a new porous synthetic polymer scaffold, Journal of Bone and Joint Surgery, 89,258-264(2007).

[104] K.D. Park, H.H. Jung, J.S. Son, J.W. Rhie, K.D. Park, K.D. Ahn, et al., Thermosensitive and Cell-Adhesive Pluronic Hydrogels for Human Adipose-Derived Stem Cells, Key Engineering Materials, 301-304(2007).

[105] D.K. Han, K.D. Ahn and S.H. Cha, inventors, Korea Institute Of Science And Technology, assignee, Injectable thermosensitivepluronic hydrogels coupled with Bioactive materials for tissue regeneration and preparation mehtod thereof, WO/2007/064152, 2007 June 7.

[106] J.H. Jeong, Y.M. Moon, S.O. Kim, S.S Yun and H.I. Shin, Human Cartilage Tissue Engineering with Pluronic and Cultured Chondrocyte Sheet, Key Engineering Materials, 89-93(2007).

[107] S. Grad, L. Kupcsik, K. Gorna, S. Gogolewski and M. Alini, The use of biodegradable polyurethane scaffolds for cartilage tissue engineering: potential and limitations, Biomaterials, 24, 5163-5171(2003).

[108] A.S. Rowlands, S.A. Lim, D. Martin and J.J. Cooper-White, Polyurethane/poly (lactic-co-glycolic) acid composite scaffolds fabricated by thermally induced phase separation, Biomaterials, 28, 2109-2121(2007).

[109] W.H. Wong and D.J. Mooney, Synthesis and properties of biodegradable polymers used as synthetic matrices for tissue engineering, in: Synthetic Biodegradable Polymer Scaffold,(ed) A. Atala and D. Mooney, Springer; 50"82(1996).

[110] N.N. Ramrattam, G.J.C. Heijkants, T.G. Van Tienen, A.J. Schouten, R.P.H. Veth and P. Buma, Assessment of tissue in growthrate in polyurethanes scaffolds in tissue engineering Tissue Engineering, Tissue Engineering, 11, 1212-1223(2005).

[111] J. Zeng, Enzymatic degradation of poly (l-lactide) and poly (epsilon-caprolactone) electrospun fibers, Macromolecular Bioscience, 12, 1118-1125(2004).

[112] M. Shin, O. Ishii, T. Sueda and J.P. Vacanti, Contractile cardiac grafts using a novel nanofibrous mesh, Biomaterials, 25, 3717“ 3723(2004).

[113] W.J. Li, R. Tuli, X. Haung, P. Laquerriere and R.S. Tuan, Multilineage differentiation of human mesenchymal stem cells in a three-dimensional nanofibrous scaffold, Biomaterials, 26 , 5158“5166(2005).