

COMMON MATERIALS FOR USING BIO TISSUE ENGINEERING

Reyhaneh Azarmi¹, Ali Ashjarian^{1*}

¹Young Researcher and Elite Club, Yadegar-e- Imam Khomeini (RAH) - Shahre-Rey Branch, Islamic Azad University, Tehran, Iran.

*Corresponding author: E.Mail: a.ashjarian@gmail.com

ABSTRACT

Many researches are presently being conducted on several different types of tissues and organs, including cartilage, Ligament, muscle, heart, blood vessels, bone, neuronal, liver etc. Bio tissue engineering is remarkably multidisciplinary, bringing together cell and molecular biologists, biochemists, engineers, pharmacologists, physicians etc. Tissue engineering aims to create new tissues and organs by introducing cells, biocompatible material and supportive factors. This review paper focuses on the using some natural and synthetic bio materials and polymers such as poly lactic-co-glycolic acid, Poly lactic acid, Poly ϵ -caprolactone, poly urethane, polyester urethane chitosan and fibrin used for tissue engineering.

Keywords: Tissue engineering, Biological, Synthetic polymers, Composite.

INTRODUCTION

For millennia, humans have been interested in manipulating the vast body of life surrounding them. As social systems and resultant technology have progressed, the motivation and ability to alter life has steadily increased. In actuality much of the biotechnology revolution that so enralls us today had its roots at the dawn of civilization. For example, humans have been practicing various forms of tissue engineering in its broadest sense for thousands of years. The manipulation of crops and domesticated animals has been a persistent and widespread practice across the planet (Herman, 2002). The simple act of the castration of a farm animal dramatically alters tissue function, yielding desired morphologic, chemical and behavioral changes. Exponential increases in our understanding of the molecular basis of cell interactions have provided the means to more precisely manipulate and perhaps duplicate tissue function. Plant tissue engineering is becoming commonplace and food, fiber, and pharmaceutical production may well be changed irrevocably (Skarja, 2001). Tissue Engineering is the in vitro development (growth) of tissues or organs to replace or support the function of defective or injured body parts or the directed management of the repair of tissues within the body (in vivo). The 20th century will be remembered as a time of revolutionary change in the fields of basic and applied science. New ideas and new tools provided both an increased understanding of the natural world as well as an unparalleled ability to alter the environment and human society (Guan, 2002).

The field of biomedicine reaped the windfall of new achievements in many science, especially improving health care in the world (Langer, 1995). Tissue engineering does open new Position in reconstructive medicine. Tissue engineered substitutes as three dimensional reconstructions can be implanted into the human body leading to rapid host implantation and acceptance (Ladd, 2009). The substitutes must have at least minimal biological and mechanical functions for such reparative role. The term reparative medicine is often used to denote the replacement repair or functional enhancement of tissues and organs. Reparative medicine has traditionally used materials at hand and the technology of the day to restore or improve function of organs and tissues afflicted with birth defects or the ravages of injury, disease, and age (Atala, 2000). The first tissue based therapies for skin grafting were developed in India around 3000 BCE, but the synthesis of substitute materials for skin and various grafting techniques (e.g., autologous and allografts) were not developed until the eighteenth century (Atala, 2006). The first engineered skin tissues were generated by Howard Green and colleagues in 1975. Tissue engineering employs aspects of molecular biology, cell biology, material technology engineering, and surgical intervention to develop tissue substitutes to restore the function and architecture of damaged or lost tissues and organs (Macchiarini, 2008).

1. TISSUE ENGINEERING

Tissue engineering is an important emerging area in biomedical engineering for creating biological alternatives for harvested tissues, implants and prostheses. In tissue engineering, a highly porous artificial extracellular matrix or scaffold is required to accommodate mammalian cells and guide their growth and tissue regeneration in three dimensions (3D). The ideal scaffold for tissue engineering should be biocompatible and biodegradable and provides sufficient and adequate interactions with various cell types. The tissue engineered scaffold should not initiate an immunological or 'foreign body response in the patient. Moreover, it should provide temporary mechanical support that

can withstand in vivo forces and maintain space for tissue development. The mechanical support should be maintained until the engineered tissue has sufficient mechanical integrity by itself. The engineered scaffold should be a provisional tissue, which facilitates in situ regeneration, thereby helping the body to heal (Langer, 1993).

2. COMMON BIO MATERIALS

There are three categories of scaffold materials in tissue engineering. Natural (biological) materials, synthetic materials and hybrid (semi-synthetic) materials naturally occurring materials have physiological activities, such as cell adhesion, mechanical properties and biodegradability (Seliktar, 2005). Moreover, cells can adhere and interact with them via integrin, followed by activation of signal transduction pathways. Natural materials can be degraded and replaced upon the generation of new tissues. Difficulties with natural materials are to obtain them with a continuous supply and immunological integrity, and to fully understand the cell-scaffold interactions. Well-investigated natural scaffolds are chitosan, fibronectin, collagen, fibrin and combinations hereof. Synthetic polymers are poly lactic-co-glycolic acid (PLGA), Poly lactic acid (PLA), and Poly ϵ -caprolactone (PCL) (Lutolf, 2005).

3. SYNTHETIC MATERIALS

3.1. Poly Lactic-Co-Glycolic Acid (PLGA): This copolymer, poly (lactic-co-glycolic acid) (PLGA) was first available as a suture material under the trade name Vicryl in 1974. PLGA scaffolds were used in the early 1990s toward engineering bone and liver and were famously used in the tissue engineering of cartilage in the shape of a human ear. PLGA in a 50:50 mixture has a degradation time of about 8 weeks. PLGA can also be blended with other polymers as well as natural materials, such as gelatin, which was used to study trabecular bone regeneration (Rosso, 2005; Alperin, 2005; Thomson, 1995; Wintermantel, 1991; Cao, 1997, Singhal, 1996).

3.2. Poly Lactic Acid (PLA): PLA is biodegradable aliphatic polyester, more hydrophobic than PGA. There are 2 racemic isoforms, poly-L-lactic acid (PLLA) and poly-D-lactic acid (PDLA). The racemic mixture can be termed poly-D,L-lactic acid (PDLLA) or simply PLA, without indication of which chiral form is present. PLA in scaffolds is usually found in a copolymer mixture (see above), although a few early studies looked at the use of PLA scaffolds for cartilage repair and nerve regeneration. PLLA fibrous scaffolds maintained integrity for a 42-day period, during which PDLLA fibrous scaffolds shrank significantly after only 3 days (Stegemann, 2007; Chu, 1995; Evans, 1999).

3.3. Poly- ϵ -Caprolactone (PCL): Poly(ϵ -caprolactone) (PCL) is a slowly degrading polymer that was first tested as a bulk material for dermal fibroblast growth. PCL scaffolds have been used toward tissue engineering efforts in bone, either alone or combined with hydroxyapatite (HA). PCL scaffolds are attractive for the longer term, as it degrades over 2 years (Li, 2006; Doyle, 1996; Corden, 2000; Calvert, 2000; Marra, 1999; Pitt, 1981).

3.4. Poly Urethane (PU): Segmented PU allow for structural variations to achieve elastomeric properties. A major limitation of PU for biomedical applications is the involvement of toxic precursors (such as toluene diisocyanates) in the synthesis. Progress has been made in the development of biodegradable PU or urethane based polymers using less toxic diisocyanates. These polymers have been explored for vascular and other tissue engineering applications (Lin, 1994; Green, 1979; Ebrod, 1981; Zhang, 2000).

3.5. Poly Glycolic Acid (PGA): PGA fibers in the forms of tassels and felts were utilized as scaffolds to demonstrate the feasibility of organ regeneration. Fiber meshes consist of individual fibers either woven or knitted into three-dimensional patterns of variable pore size. The advantageous characteristic features of fiber meshes are a large surface area for cell attachment and a rapid diffusion of nutrients in favor of cell survival and growth. PGA is simple, linear, aliphatic polyester that was first used as a biodegradable suture. The PGA suture was brought to market under the trade name Dexon. PGA in scaffolds was first introduced in the 1980s, alone as a mesh to investigate renal injury (McAninch, 1986). Blended Dacron (polyethylene terephthalate), to study tendon and ligament repair (Cabaud, 1982; Rodkey, 1985; Townley, 1985). Large-scale production of fibrous PGA scaffolds with consistent porosity was achieved in the early 1990s, which was used to regenerate cartilaginous tissue (Freed, 1994). The degradation rate was studied in vitro, whereby only 30% of the polymer remained after 8 weeks (Saad, 1997).

3.6. Degrapol: DegraPol is a polyester-urethane; it consists of two blocks of polymers which impart very different physical and mechanical properties to the final product. It consists of polyhydroxybutyrate diol (Hard Segment) and polycaprolactone-dyglycol-diols (Soft Segment). Both are biodegradable polymers and their degradation products are not toxic. Using various ratios of hard and soft segments it's possible to modulate the mechanical properties of the final

product. Unlike traditional materials, DegraPol shows a broad range of elastic modulus, making it a potential new material for the regeneration of many types of biological tissues (Woo, 2007).

3.7. Hydroxyapatite (HAP): The HAP-containing scaffolds improve osteoplastic cell seeding uniformity and show significantly enhanced expression of mature bone marker genes such as osteocalcin and bone sialoprotein over plain polymer scaffolds. Bone tissue formation throughout the scaffold has been demonstrated. Our recent data indicate that HAP in the composite scaffolds. Significantly improves the protein adsorption capacity, suppresses apoptotic cell death and provides a more favorable microenvironment for bone tissue regeneration (Lee, 2000).

3.8. Phosphate (CAP): Substantial progress has been made in the analysis of progenitor cells with regard to differentiation pathways. This knowledge is being incorporated into the design of future scaffolds particularly with regard to optimization. Biomaterial development and final design will be essential to the appropriate stimulation and differentiation of bone cells. The environment in which these CaP tissue-scaffold systems are cultivated will greatly affect the long-term tissue viability. However, the diverse nature and independent processing parameters of research in this field makes comparisons especially difficult and the need for consistency fundamental. As such, standardization will hopefully expedite the development of successful tissue-engineering alternatives (Yu, 2010).

4. NATURAL MATERIAL

4.1. Chitosan: The natural biopolymer chitosan is an excellent candidate for the preparation of wound dressings and hydrogel scaffolds for tissue engineering. There are different ways to form hydrogels from chitosan. Chitosan could be used alone but this is rarely the case because pure chitosan hydrogel is fragile and has low mechanical strength, which limits its application in tissue engineering. Chitosan has therefore been combined with other compounds or chemically modified to improve its properties for tissue engineering applications, in particular to create thermo sensitive hydrogels that will gel in situ (Wood, 2008).

4.2. Collagen: Collagen is the most widely distributed class of proteins in the human body. Collagen can be extracted from various sources considering that it is one of the most abundant proteins on earth. It can be extracted from almost every living animal, even including alligators and kangaroos. The use of collagen-based biomaterials in the field of tissue engineering applications has been intensively growing over the past decades. Collagen possesses a major advantage in being biodegradable, biocompatible, easily available and highly versatile. However, since collagen is a protein, it remains difficult to sterilize without alterations to its structure (Kellner, 2001).

4.3. Fibrin: There are a number of commercially available fibrin products with different amounts and origins of the components (Rotter, 2002; Ma, 2001). The concentration of fibrinogen, varying between 40 and 125 mg/ml, is directly correlated to the tensile strength of the fibrin clot, whereas the degree and speed of clotting. The latter proves useful for quick haemostasis to prevent blood loss. (e.g, in suturing of vessels) or in surgical procedures involving careful glue adjustment to fit a tissue or organ (Wang, 2001). Within 3 days of application, a preliminary granulation tissue with a large number of wound healing cells is present and is subsequently replaced with collagen fibers one to two weeks later. During normal wound healing the fibrin glue is absorbed within days to weeks depending on the type of sealant and location of application (Webster, 2000).

4.4. Ceramic: Ceramics have been used in bone tissue engineering due to their osteoinductive and biocompatible properties (Zhou, 2007). Nanophase ceramics - sans immobilized peptides seeded with osteoblasts as osteointegrative devices designed to merge with apposed bone, have come to the fore as well⁽⁴⁷⁾. The absence of peptides on the nanophase ceramics would circumvent the problems which could arise from the interactions of the peptides with the biomaterials. For instance, nanophase alumina, titania, zirconia, and hydroxyapatite (HA), with grain sizes less than 100nm, were compared with their conventional counterparts in regards to cell proliferation, cell adhesion, matrix formation, cellular migration and cell differentiation (Boccaccini, 2003).

4.5. Hydrogels: Hydrogels have many different applications in the field of regenerative medicine. Biodegradable, injectable hydrogels could be utilized as delivery systems, cell carriers, and scaffolds for tissue engineering. Injectable hydrogels are an appealing scaffold because they are structurally similar to the extracellular matrix of many tissues, can often be processed under relatively mild conditions and may be delivered in a minimally invasive manner. Injectable hydrogels are promising substrates for tissue engineering applications due to high tissue like water content, ability to homogeneously encapsulate cells, efficient mass transfer, easily manipulated physical properties and minimally invasive delivery (Tememoff, 2000; Caldwell, 1997; Breen, 2009).

5. HYBRID (SEMI- SYNTHETIC) MATERIALS

5.1. Polymethyl Methacrylate (PMMA): PMMA is biocompatible in the human eye that the idea of an artificial cornea resurfaced. Unfortunately, biocompatibility was not sufficiently acceptable for a good artificial cornea, because keratoprostheses made from PMMA had a problem of extrusion. Many including Gigard and Cardona, have tested different design and materials, however, long term retention remains a major problem (Kaufman, 1998; Pitt, 1981).

6. CONCLUSION

In the preceding paper, a framework has been sketched to view introduction of bio tissue engineering and categories of scaffold materials in tissue engineering. Tissue engineering aims to develop functional substitutes for damaged or diseased tissues through complex constructs of living cells, bioactive molecules, and 3D porous scaffolds that support cell attachment, proliferation, and differentiation. Tissue engineering experienced an exponential growth during the last decade from an emerging conceptual stage into a fast developing and multifaceted field. Due to the intrinsic interdisciplinary and multidisciplinary nature of this field, the fast evolution and development of the field of tissue engineering have benefited from the convergence of the progresses in each and every area of the field. Application of bio tissue engineering are improve of renal injury, regenerate cartilaginous, bone, liver, nerve regeneration, dermal fibroblast growth and Endocrinology vascular.

REFERENCES

- Alperin C., Zandstra PW., Woodhouse KA, Polyurethane films seeded with embryonic stem cell derived cardiomyocytes for use in cardiac tissue engineering applications, *Biomaterials journals.*, 26(73), 2005, 77–86.
- Atala A, Tissue engineering of artificial organs, *J. Endourol.*, 14(1), 2000, 49–57.
- Atala A., Bauer SB., Soker S., Yoo JJ., Retik AB, Tissue-engineered autologous bladders for patients needing cystoplasty, *Lancet.* 367, 2006, 1241–1246.
- Boccaccini AR., Maquet V., 2003. 'Bioresorbable and bioactive polymer/Bioglass® composites with tailored pore structure for tissue engineering applications'. *Compos. Sci. Technol.* 16(63), 2003, 2417-2429.
- Breen A., O'Brien T., Pandit A, Fibrin as a Delivery System for Therapeutic Drugs and Biomolecules, *Tissue Eng Part B Rev*, 15(2), 2009, 201-214.
- Cabaud HE., Feagin JA., Rodkey WG, Acute anterior cruciate ligament injury and repair reinforced with a biodegradable intraarticular ligament, *Am J Sports Med.* 10(5), 1982, 259-265.
- Caldwell DR, The soft keratoprosthesis, *Trans am Ophthalmol Soc.*, 95, 1997, 751-802.
- Calvert JW., Marra KG., Cook L., Characterization of osteoblast-like behavior of cultured bone marrow stromal cells on various polymer surfaces, *Biomed Mater Res.* 52(2), 2000, 279-284.
- Cao Y., Vacanti JP., Paige KT, Transplantation of chondrocytes utilizing a polymer-cell construct to produce tissue-engineered cartilage in the shape of a human ear'. *Plast Reconstr Surg.* 100, 1997, 297–302.
- Chu CR., Coutts RD., Yoshioka M, Articular cartilage repair using allogeneic perichondrocyte-seeded biodegradable porous polylactic acid (PLA): a tissue engineering study, *J Biomed Mater Res.*, 29(9), 1995, 1147–54.
- Corden TJ., Jones IA., Rudd CD., Physical and biocompatibility properties of poly-epsilon-caprolactone produced using in situ polymerisation: a novel manufacturing technique for long-fibre composite materials'. *Biomaterials.*, 1(7), 2000, 13–24.
- Doyle V., Pearson R., Lee D., An investigation of the growth of human dermal fibroblasts on poly-L-lactic acid in vitro'. *J Mater Sci Mater Med.* 6(7), 1996, 381–385.
- Ebrod A, Grafting of burns with cultured epithelium prepared from autologous epidermal cells, *Lancet.* 1(8211), 1981, 75-78.
- Evans GR., Brandt K., Widmer MS, In vivo evaluation of poly (L-lactic acid) porous conduits for peripheral nerve regeneration'. *Biomaterials*, 20(12), 1990, 1109–15.
- Freed LE, Vunjak-Novakovic G., Biron RJ, Biodegradable polymer scaffolds for tissue engineering'. *Biotechnology*, 12(7), 1994, 689-93.
- Green H., Kehinde O., Thomas J, Growth of cultured human epidermal cells into multiple epithelia suitable for grafting'. *Proc Natl Acad Sci U S A.* 76(11), 1979, 5665–5668.

- Guan JJ, Sacks MS., Beckman EJ., Wagner WR, Synthesis, characterization, and cytocompatibility of elastomeric, biodegradable poly(ester-urethane) ureas based on poly(caprolactone) and putrescine, *Journal of Biomedical Materials Research*, 3(61), 2002, 493–503.
- Herman AR, The history of skin grafts, *J Drugs Dermatol.*, 1, 2002, 298–301.
- Kaufman He., Barron BA, McDonald MB, The cornea .2nd end, Newton, MA Butterworth Heinemann. Pitt C G., Chasalow F I., Hibionada Y M., et al. 1981. 'Aliphatic polyesters. 1. The degradation of poly(epsilon-caprolactone) in vivo'. *J Appl Polym Sci.*, 26, 1981, 3779–87.
- Kellner K., Schulz M B., Gopferich A., Blunk T, Insulin in tissue engineering of cartilage :a potential model system for growth factor application, *J Drug Target*.6, 2001, 439-448.
- Ladd M R., Lee S J., Atala A., Yoo J J, Bioreactor maintained living skin matrix'. *Tissue Eng.* 15(4), 2009, 861–868.
- Langer R., Vacanti J P, *Tissue engineering. Science*, 260, 1993, 920–926.
- Langer R., Vacanti J P., Vacanti C A., Atala A., Freed L E., Vunjak- Novakovic G, *Tissue engineering: biomedical applications*, *Tissue Eng.* 1, 1995, 151–161.
- Lee F Y., Hazan E J., Gebhardt M C., Mankin H J., Experimental Model for Allograft Incorporation and Allograft Fracture Repair, *J. Orthop. Res.* 18(2), 2000, 303-306.
- Lee K Y., Mooney D J, Hydrogels for tissue engineering, *Chem. Rev.* 101(7), 2001, 1869–1879.
- Li W J., Cooper J A., Mauck R L., Fabrication and characterization of six electrospun poly(alpha-hydroxy ester)-based fibrous scaffolds for tissue engineering applications, *Acta Biomater.* 2(4), 2006, 377–385.
- Lin H B., Sun W., Mosher D F., Garcia-Echeverria C., Schaufelberger K., Lelkes P I., Cooper S L, Synthesis. Surface, and cell-adhesion properties of polyurethanes containing covalently grafted RGD peptides, *J Biomed Mater Res.* 28(3), 1994, 329-342.
- Lutolf M P., Hubbell J A, Synthetic biomaterials as instructive extracellular microenvironments for morphogenesis in tissue engineering, *Nat Biotechnol.*, 23(1), 2005, 47-55.
- Ma P X., Zhang R., Xiao G., Franceschi R, Engineering new bone tissue in vitro on highly porous poly (alpha-hydroxyl acids)/hydroxyapatite composite scaffolds, *J Biomed Mater Res.*, 54(2), 2001, 284–293.
- Macchiarini P, Transplantation of a tissue-engineered airway, *Lancet journal.* 372, 2008, 2023–2030.
- Marra K G., Szem J W., Kumta P N, In vitro analysis of biodegradable polymer blend/hydroxyapatite composites for bone tissue engineering'. *J Biomed Mater Res*, 47(3), 1999, 324-35.
- McAninch J W., Schmidt R A, Polyglycolic acid mesh in repair of renal injury, *Urology.*, 28, 1982, 127–130.
- Pitt C G., Gratzl M M., Kimmel G L, Aliphatic polyesters. 2. The degradation of poly(DL-lactide), poly(epsilon-caprolactone), and their copolymers in vivo, *Biomaterials.*, 2(4), 1981, 215–220.
- Rodkey W G., Cabaud H E., Feagin J A, A partially biodegradable material device for repair and reconstruction of injured tendons. Experimental studies, *Am J Sports Med.*, 13(4), 1985, 242-247.
- Rosso F., Marino G., Giordano A., Barbarisi M., Parmeggiani D., Barbarisi A, Smart materials as scaffolds for tissue engineering'. *J Cell Physiol.*, 203(3), 2005, 465-470.
- Rotter N., Bonassar L J., Tobias G., lebl M., Roy A k., Vacanti C A, Age dependence of biochemical and biomechanical properties of tissue-engineering human septal cartilage. *Biomaterials*, 23(15), 2002, 3087-3094.
- Saad B., Keiser O.M., Welti M., Uhlschmid G.K., Neuenschwander P., Suter U W, Multiblock copolyesters, *J Mater Sci Mater Med.*, 8(8), 1997, 497-505.
- Seliktar D, Extracellular stimulation in tissue engineering, *Ann N Y Acad Sci.*, 1047, 2005, 386-394.
- Singhal A R., Agrawal C M., Athanasiou K A, Salient degradation features of a 50:50 PLA/PGA scaffold for tissue engineering'. *Tissue Eng.*, 2, 1996, 197–207.
- Skarja GA., Woodhouse KA, In vitro degradation and erosion of degradable, segmented polyurethanes containing an amino acid-based chain extender, *Chem. Pharm. Bull.*, 12(8), 2001, 51–73.
- Stegemann J P., Kaszuba S N., and Rowe S L, Review: advances in vascular tissue engineering using protein based biomaterials'. *Tissue Eng.*, 13(11), 2007, 2601-2613.
- Tememoff J S., Mikos A G., 2000. 'Injectable biodegradable materials for orthopedic tissue engineering. *Biomaterials*'. 21(23):2405–2412.
- Thomson R C., Yaszemski M J., Powers J M, Fabrication of biodegradable polymer scaffolds to engineer trabecular bone'. *J Biomater Sci Polym Ed.*, 7, 1995, 23–38.
- Townley C O., Fumich R M., Shall L M., 1985. 'The free synovial graft as a shield for collagen In growth in cruciate ligament repair'. *Clin Orthop Relat Res.*, 197, 1985, 266–271.
- Wang L, Tissue engineering the mandibular condyle. *Tissue Engineering*'. 13(8), 2007, 1955-1971.

- Webster T J., Ergun C., Doremus R H., Siegel R W., Bizios R, Enhanced functions of osteoblasts on nanophaseceramics. *Biomaterials*, 21(17), 2000, 1803-1810.
- Wintermantel E., Cima L., Schloo B, Angiopolarity: a new design parameter for cell transplantation devices and its application to degradable systems'. *ASAIO Trans.*, 37(3), 1991, 334–6.
- Woo K M., Seo J., Zhang R., Ma P X, Suppression of apoptosis by enhanced protein adsorption on polymer/hydroxyapatite composite scaffold'. *Biomaterials.*, 28(16), 2007, 2622–2630.
- Wood A., Ogawa M., Portier R J., Schexnayder M., Shirley M., Losso J N, Biochemical properties of alligator (*Alligator mississippiensis*) bone collagen'. *Comp. Biochem. Physiol. B Biochem. Mol. Biol*, 151(3), 2008, 246–249.
- Yu AQi B., The preparation and cytocompatibility of injectable thermo sensitive chitosan/poly(vinyl alcohol) hydrogel'. *J HuazhongUnivSciTechnolog Med Sci.*, 30(1), 2010, 89-93.
- Zhang J Y., Beckman E J., Piesco N P., Agarwal S, A new peptide-based urethane polymer: synthesis, biodegradation, and potential to support cell growth in vitro, *Biomaterials.*, 12(21), 2000, 1247–1258.
- Zhou G S., Su Z Y., Cai Y R., Liu Y K., Dai L C., Tang R K, Different effects of nanophase and conventional hydroxyapatite thin films on attachment, proliferation and osteogenic differentiation of bone marrow derived mesenchymal stem cells'. *Bio-Medical Materials and Engineering*, 17(6), 2007, 387-395.