Journal of Scientific and Innovative Research 2014; 3 (2): 265-272
**Available online at: www.jsirjournal.com**


**Research Article**

ISSN 2320-4818
JSIR 2014; 3(2): 265-272

Received: 06-03-2014
Accepted: 25-04-2014

**Jebahi S**
*Animal Ecophysiology Laboratory, Sfax Faculty of Science, Department of Life Sciences, Sfax, Tunisia*

**Farhat L**
*Service of Medical Oncology, CHU Habib Bourguiba, 3029 Sfax, Tunisie, Sfax, Tunisia*

**Oudadesse H**
*University of Rennes 1, UMR CNRS 6226, Campus de Beaulieu, 263 av. du Général Leclerc, 35042 Rennes, France*

**Rebai T**
*Histology, Orthopaedic and Traumatology laboratory, Sfax Faculty of Medicine, Sfax, Tunisia*

**El Feki A**
*Animal Ecophysiology Laboratory, Sfax Faculty of Science, Department of Life Sciences, Sfax, Tunisia*

**Jamel Doued**
*Service of Medical Oncology, CHU Habib Bourguiba, 3029 Sfax, Tunisie, Sfax, Tunisia*

**Hassib Keskes**
*Histology, Orthopaedic and Traumatology laboratory, Sfax Faculty of Medicine, Sfax, Tunisia*

**Correspondence:**
**Samira Jebahi**
University of Rennes 1, UMR CNRS 6226, Campus de Beaulieu, 263 av. du Général Leclerc, 35042 Rennes, France
**E-mail:** jbahisamira@yahoo.fr

# Molecular biological and advances in materials

*Jebahi S*, Farhat L, Oudadesse H, Rebai T, El Feki A, Jamel Doued, Hassib Keskes*

## Abstract

The past twenty years have witnessed a revolution in biomedical research, with the advent and rapid expansion of cell and molecular biology. These Advances have been applied to the biomaterials field. This paper aims to review the progress in biomaterials and the several therapeutic strategies in order to examine biomaterial interactions at the cellular and the wider host level. The performance of any materials as carriers of different types of drugs, such as antibiotics, as well as growth factors with the express intention of delivering some biologically or pharmacologically active agent is also reviewed. The current status of how protein, cell and gene therapy used to modify the properties of the biomaterial to advance the art of tissue engineering is also updated. Moreover, the favorable properties of nanotechnology exploited in biomedical applications were widely focused on. In our opinion, these advances represent some of the few examples in which the progress of molecular biology has a good chance of early clinical success.

**Keywords:** Therapeutic strategies, Factor growth, Nanotechnology, Gene Delivery, cell interaction.

## Introduction

The development of biomaterials for tissue engineering applications has recently focused on the design of biomimetic materials that are able to interact with surrounding tissues by Biomolecular recognition.[1] It is noted that in recent times, various groups in the biomaterial research field have adopted molecular biological techniques to improve understanding of tissue -biomaterial interactions. In this field, extensive studies have been performed to render materials biomimetic. Previous work has used long chains of extracellular matrix (ECM) proteins such as fibronectin (FN), vitronectin (VN), and laminin (LN) for surface modification.[2] Biomaterials can be coated with these proteins, which usually promotes cell adhesion and proliferation.[3] The molecular biology revolution and the advances of the 1970s led to a pronounced increase in the efficacy of biomaterials.The advances in genomics and proteomics in the 1990 and 2000 significantly affected the ways in which biomaterials are designed and used (Figure 1).[4] On the other hand, nanotechnology, which first appeared in the twentieth century, is an area of science devoted to the manipulation of materials atoms and molecules of materials in the nanometer range. Therapeutic uses of nanoparticles are among the most recent developments, including all particles that possess at least one dimension that is less than 100 nm. It has been shown that the remarkable recognition capabilities of biomolecules, when combined with the unique properties of nanomaterials can lead to novel tissue substitutes, biological electronics such as biosensors, sensitive diagnostic

systems, and controlled drug delivery systems with significantly improved performances. The purpose of the present paper is to review the cell and molecular biological approach for biomaterial research perfection. The important aspects of interfacial biology, including the underlying biological mechanisms and methodology are presented.

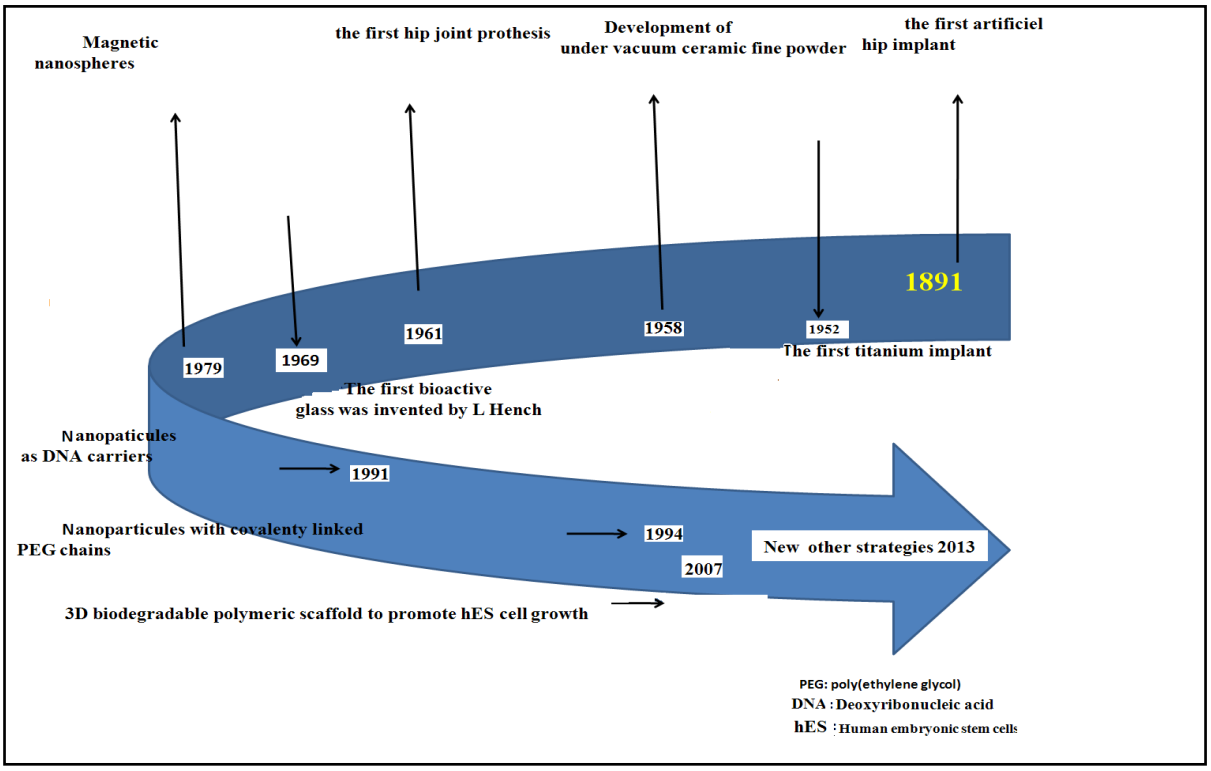


**Figure 1:** Development of Biomaterials for tissue engeneering

## Advances in biomaterials

It is encouraging to note that in recent years, various teams in the biomaterial research field have adopted molecular biological techniques to improve understanding of tissue-biomaterial interactions.

## The growth factors and other proteins

The term "growth factor" refers to a naturally occurring protein capable of stimulating cellular proliferation and differentiation. Growth factors are important for regulating a variety of cellular processes (Figure 2). They act as signaling molecules between cells and are involved in neuromodulation. These molecules bind to specific receptors on the surface of their target cells. They often promote cell differentiation and maturation. Growth factors are acting on cell-surface receptors and directing cellular activities involved in wound healing. Bone morphogenetic proteins (BMPs, particularly BMP-2and-7), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), and fibroblast growth factor (FGF, essentially bFGF, also known as FGF-2) are a naturally occurring substance capable of stimulating cellular growth proliferation and cellular differentiation.[5] Among the most fertile areas of research progress, we can cite the controlled delivery of platelet-rich plasma-derived PRP growth factors for bone formation. The bioavailability of the PRP-derived growth factors using a hydrogel carrier system were used in some studies.[6] Ever since, it was initially reported in 1980s, PRP quickly caught the attention of clinicians for its easy obtainability, autologous origin, safety, cost-effectiveness, and functions in promoting both angiogenesis and bone regeneration. When injected percutaneously into nude mice, there were no signs of rejection, infection, or skin necrosis during the whole inspection period. At 1 and 3 months, only 10% PRP-ADSC-laden microspheres (group IV) and 15% PRP-ADSC-laden microspheres (group V) hardened at the harvest, presented as solid clumps with white calcified matrix. The other three groups (groups I, II, III) remained as semi translucent gels and were barely visible under the micro-CT scanning of platelet-rich plasma (PRP) and adipose derived stem cells (ADSCs). Improving

vascularization of tissue-engineered bone can enhance cell performance in vivo and further promote bone regeneration. It was hypothesized that a mixture of platelet-rich plasma (PRP) and adipose derived stem cells (ADSCs) could endure the alginate microspheres with osteogenic and angiogenic potential. In one strategy, bioactive glass was functionalized, with collagen. The stable collagen attachment to the functionalized 45S5 Bioglass®-based porous scaffolds make this approach potentially suitable for improving cell attachment and thus for enhancing the application potential of the scaffold in tissue engineering. A highly-interconnected porous scaffold made from 45S5 Bioglass® was fabricated by the polymer replica technique and surface functionalized for protein immobilization.[7] It was discovered that the surface functionalization enhanced the stability of the collagen attachment and the stability against the pH increment in a biological environment. The growth factors and other proteins have the ability to promote the regeneration of tissues in the locomotive system, but their clinical use is often hindered by delivery problems.[8] In principle, these problems can be overcome by delivering the relevant genes, as the therapeutic substances, thereby can be persistently produced directly by local cells at the disease site. In fact, following genetic modification, they are capable of sustained expression of transgenic products at biologically relevant levels. Genetic engineering may improve on natural proteins for applications in tissue engineering.

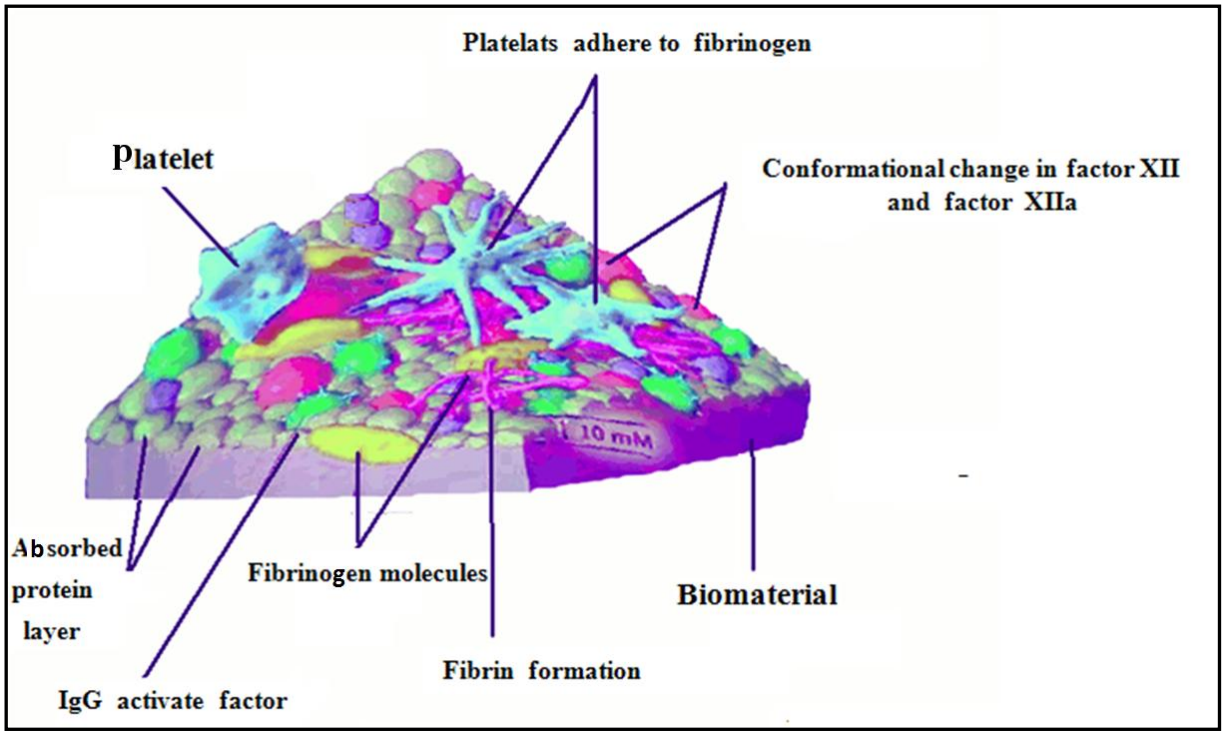


**Figure 2:** Biomaterial interaction with the physiological environment

**Molecular and gene delivery**

In general, the molecular biology revolution advances in genomics and proteomics in the gene therapy involve the transfer of genetic information to the cells. When a gene is transferred to a target cell, the cell synthesizes the protein encoded by the gene.[9, 10] For gene expression, the transferred DNA material must enter the nucleus where it can be transcripted. After transcription, the generated m-RNA is transported outside the nucleus and serves as a matrix for the production of proteins in the ribosome. The gene can be introduced directly to a specific anatomic site (in-vivo technique) or specific cells can be harvested from the patient, expanded, genetically manipulated in tissue culture and then reimplanted (ex-vivo technique).[11] Current efforts in the area of drug delivery were developed in order to achieve efficient targeted delivery. The local delivery strategy was used, for example for fracture healing in the context of osteoporosis.[12] In one strategy, calcium-based injectable bone cement clinically was applied to inhibit osteoclast-induced bone resorption.[13] This strategy consisted in the RNA delivery interfering technology to bone sites to target and down-regulate osteoclast.[14] In fact, polymer poly

(lactic-co-glycolic acid) (PLGA) microparticles were exploited as a passive phagocyte-targeting carrier to deliver RANK siRNA to both osteoclast precursors and osteoclasts - the professional phagocytes in bone. These natural phagocytes internalized micron-sized particles, while most other non-targeted cells in bone could not PLGA-siRNA microparticles were dispersed within the biomedical grade calcium-based injectable bone cement clinically used in osteoporosis as a bone augmentation.[15] In another study, we designed a novel gene-activated matrix (GAM) with embedded Chitosan/plasmid nanoparticles encoding platelet derived growth factor (PDGF) based on porous Chitosan/collagen composite scaffold. GAM blends two strategies, serving as a local bioreactor with therapeutic gene expression and providing a structural template to fill the lesion defects for cell adhesion, proliferation and synthesis of extracellular ECM.[16] The chitosan/collagen scaffold acted as a three-dimensional carrier and chitosan nanoparticles condensed plasmid DNA.[17] Moreover, some biomaterials such as calcium carbonate ($CaCO_3$) microparticles were for the first time used for efficient delivery of p53 gene to transfect human cancer cells HeLa. $CaCO_3$ microparticles (2–4μm) absorbed pEGFP-C1-p53 (expressing the GFP-P53 fusion protein) to transfect HeLa cells. Flow cytometer (FCM) was used to evaluate the gene transfection efficiency in these cells, which were stably transduced with a green fluorescent protein gene.[18] In this study, $CaCO_3$ delivering pEGFP-C1-p53 could transfect about 5% of the tumor cells in culture. However, the efficiency rate of tumor cell apoptosis was surprisingly up to 80%. Meanwhile, the results of MTT assay and crystal violet staining showed that the CaCO3microparticles had low Cytotoxicity.[19] In this case, no appreciable cytotoxic effects were found on any of the four cell lines. Viral and non-viral vectors can also be used for the delivery of genetic materials into cells. Non-viral gene transfer systems, such as liposomes, naked DNA are usually easier to produce and to have a lower toxicity and immunogenicity, but the efficiency of their gene delivery is impeded by a blow rate of infection unless the transduced cells are selected.[20] Recently, viral gene vectors, including retrovirus, adenovirus, adeno-associated virus and herpes virus, are considered as the most efficient method of gene transfer.[21]

**Nanobiomaterial**

The nanomaterials level is the most advanced at present, both in scientific knowledge and in commercial applications. A decade ago, nanoparticles were studied because of their size-dependent physical and chemical properties.[22] Now they have entered a commercial exploration period.[23, 24] Nanomaterials have unique surface properties and energetics due to higher surface areas, higher surface roughness, higher amounts of surface defects (including grain boundaries), altered electron distributions, etc. All of these unusual properties inherent of nanomaterials will affect interactions with proteins since all proteins are nanoscale entities. Recently, Cheng *et al*. reported that human cartilage cells attached and proliferated well on HA nanocrystals homogeneously dispersed in polylactic acid (PLA) composites.[25] Matthews et al. demonstrated that type II of collagen could be electrospun to form nonwoven fibrous scaffolds with fiber diameters ranging from 110 nm to 1.8 μm to support chondrocyte growth and infiltration.[26] Even anodized metals (such as Ti) possessing nanometer pore increase chondrocyte adhesion and migration.[27] In a very enhanced strategy of biomaterial use, nanoparticles are being designed to exploit the chemical and physical differences between normal and tumour-associated vasculature in order to concentrate the particles selectively within or near tumours, allowing subsequent drug induced cell death. Recent demonstrations of polymeric nano particles could be designed for trafficking through the lymphatic vessels to target T cells in the lymph nodes. The synergistic enhanced effect of the relevant gold nanoparticles on the drug uptake of target cancer cells could provide a new strategy to inhibit the multidrug resistance of the respective cancers.[28, 29] Magnetic nanoparticles were also used for improving cell invasion in tissue engineering. Ion flows caused by electromechanical stimulation could probably modulate regeneration, suggesting that electrochemical signals could be used to alter cell fate directly and by manipulating biomaterial structure and presentation of chemical epitopes indirectly.[30] In another study, PMMA-based cements containing magnetite (C-PMMA/$Fe_3O_4$) was found in 9 be useful in hyperthermia treatment for bone tumor. These investigations are useful for designing new PMMA/$Fe_3O_4$ bone cement with high heating efficiencies and biocompatibilities for bone tumor treatments. C-PMMA/$Fe_3O_4$ was prepared by incorporating $Fe_3O_4$ powders of different diameters (means of 300, 35, and 11 nm) into the polymerization reaction of PMMA to develop new bone cement with high heating efficiencies in alternating current magnetic fields.[31] The

in vitro heating capability of the cements in different magnetic fields was investigated. The heat generation strongly depended on the magnetite nanoparticle sizes and applied magnetic fields. The incorporation of $Fe_3O_4$ into cement 30 wt % of the total amount of cement did not significantly change the cement compressive strength of, and the proliferation of rat fibroblast Rat-1 cells on cement discs was not inhibited.[32] Furthermore, nanodiamond (ND) which is a carbon nanomaterial developing for biological applications in recent years was studied. In this study, we investigated the location and the distribution of 100 nm carboxylated ND particles in cell division and differentiation.[33] ND particles were taken into cells by macropinocytosis and clathrin-mediated endocytosis pathways. However, the cell growth ability was not altered by endocytic ND particles after long-term cell culture for 10 days in both A549 lung cancer cells and 3T3-L1 embryonic fibroblasts. ND particles were equal separating into two daughter cells by cell division approximately. Finally, the cell retained a single ND's cluster in cytoplasm after being subculture for several generations. Interestingly, ND's clusters were carried inside of the cell but without inducing damage after long-term cell culture. Moreover, ND particles did not interfere with the gene or protein expressions on the regulation of cell cycle progression and adipogenic differentiation. Together, these findings provide that endocytic ND particles are non-cytotoxic in cell division and differentiation, which can be applied for the labeling and tracking of cancer and stem cells. Pharmacodynamic studies were also interested in the nanoparticule system.[34] In fact, some copolymeric nanocarriers were used like as product 10 drug vehicles assembled by amphiphilic polyphosphazene bearing poly(Nisopropylacrylamide) (PNIPAAm) and ethyl glycinate (EtGly) as substitutes.These complexes were investigated as drug vehicles for indomethacin (IND).[35]

**Advances in the Technical analysis**

From a cell biology perspective, 2D cell culture models only provide physiologically compromised cells induced by an unnatural environment[36], and the lack of 3D structure will cause cells to form a random 2D mono-layer.[37] In vivo, cells are subjected to growth in three dimensions and complex cell-cell interactions. This observation encouraged a paradigm shift from conventional 2D cell culture models towards 3D microenvironments.[38] To obtain a more realistic understanding of cell- cell and cell- biomaterial interactions, Kirkpatrick *et al*. proposed the use of co-culture models in vitro.[39] Other techniques were largely used for the evaluation of cell-materials interaction. For example, histology, was used to search for qualitative differences. Before and after cellular interaction with biomaterial, histological investigations suffer several limitations. The primary challenge is one of tissue preservation, as fixation and tissue dehydration will affect the phenotype of the cells, possibly altering their interpretation. Other challenges include staining variability and the subjective opinions of the pathologist that scores the stained tissue sections.[40] Improvements came in the early 1990s with the aid of computerized image analysis tools. Based on immunohistochemical (IHC) staining with monoclonal antibodies specific for a subset specific cell type, originally inflammatory cells.[41] For example Analysis HL-60 was used as a model of the macrophages. The expression of interleukin-1β (IL-1β) mRNA, a cytokine secreted by macrophages, was selected to estimate the extent of inflammation. The expression of IL-1β mRNA in the HL60 cells cultured on various substrates and in various conditions was studied. Expression of IL-1β could be successfully determined by RT-PCR analysis. Northern blot analysis and reverse transcription polymerase chain reaction (RTPCR) assays are used to study mRNA expression through the isolation and profiling of a specific mRNA (or a discrete number of transcripts) in a sample.[42] The DNA microarrays have become standard, powerful tools to investigate global gene expression patterns. Microarrays have been used in one study to investigate the changing environments in response to different types of biomaterials including metals, titanium, polymers and textile/fibrin gels. These responses extend to genes encoding ECM proteins[43], cellular adhesion molecules and proteins involved in inflammation and apoptosis.[44] The use of microarrays to profile the transcriptome in the context of biomaterial interactions, has continued to provide extensive molecular insights. As reported, DNA microarray technology was used in one study, to assess both the cytotoxicity and global gene interaction between 100 μm and 200 μm nickel ions (Ni2+) and mouse fibroblast cells (L929) over 4 time points (12 h, 24 h, 48 h & 72 h).[45] Nickel–titanium (NiTi) is widely used in the medical field, including in orthodontics, root canals and colon surgery. These techniques are useful for targeted experiments; for example, they have been used to assess the relationship between transforming growth factor-beta 1(TGF-β1) and the mineralisation of an in vitro osteoblast/implant culture system.[46] However,

exploring the molecular environment and the genes which regulate this communication could help in the early response prediction and in profiling fundamental positive and negative responses. For example, AuNPs in bulk are chemically inert with no inherent toxicity. However, exploring the gene expression patterns of accumulated AuNPs in liver and spleen of rat models resulted in 79 and 62 differentially expressed genes, respectively, and 10 genes in common between the two organs. Classification of these genes included several pathways, including detoxification, lipid metabolism, cell cycle, defense response and circadian rhythm. This datum demonstrates that the AuNPs are not entirely biologically inert and is an important consideration for the application of these materials.

## Conclusion

Many experts agree that the greatest hope for treatment of the damaged tissues will involve a combinatorial approach that integrates biomaterial scaffolds, cell transplantation, and molecule delivery. This continuing research progresses permit the discovery of new biomaterials that may provide yet another opportunity to address clinical needs. Among the most progressive strategies, localized delivery of growth factor is believed to be therapeutically effective for cellular component replication involved in tissue development and the healing process. Likewise, gene therapy has the potential to treat a disease by replacing, altering, or supplementing a gene that is either absent or abnormal, a condition that is responsible for that disease. Moreover, the nanotechnology has distinct properties that make the nanostructures potential candidates for different nanoscale bio-medical and sensor device applications. All these applications include the design of product formulations with improved drug delivery performance, or the modification of implant surfaces for enhancing cell attachment and proliferation have known a great progress to improve tissue-biomaterial interactions.

## References

1. Jandt .K.D. Evolutions, Revolutions and Trends in Biomaterials Science - a Perspective. Adv Eng Mat.9, 2007: 1035–1050.

2. P. B. van Wachem, C. M. Vreriks, T. Beugeling, J. Feijen, A. Bantjes, J. P. Detmers, and W. G. van Aken. The influence of protein adsorption on interactions of cultured human endothelial cells with polymers Journal of Biomedical Materials Research, 1987; 21, 701-718.

3. Chang DT, Jones JA, Meyerson H, Colton E, Kwon IK, Matsuda T, Anderson JMJ. Lymphocyte/macrophage interactions: biomaterial surface-dependent cytokine, chemokine, and matrix protein production. Biomed Mater Res A. 2008 1;87:676-687.

4. Huebsch N, Mooney DJ. Inspiration and application in the evolution of biomaterials. Nature 2009; 26: 426-32.

5. Lim, S. M., Jang, S. H., Oh, S. H., Yuk, S. H., Im, G. I. and Lee, J. H. Dual-Growth-Factor-Releasing PCL Scaffolds for Chondrogenesis of Adipose-Tissue-Derived Mesenchymal Stem Cells. Adv. Eng. Mater. 2009; 1-2:B62-B69.

6. Lin SS, Landesberg R, Chin HS, Lin J, Eisig SB, Lu HH.. Controlled release of PRPderived growth factors promotes osteogenic differentiation of human mesenchymal stem cells. Conf Proc IEEE Eng Med Biol Soc 2006; 1:4358-4356.

7. Varkey M,Gittens SA, Uludag H. Growth factor delivery : anupdate. Expert Opin Drug Deliv 200 2004;1:19e36.

8. Aslam S, Barbara W. Trautner, Venkat Ramanathan, Rabih O. Darouiche. Combination of Tigecycline and N-Acetylcysteine Reduces Biofilm-Embedded Bacteria on Vascular Catheters . Antimicrob Agents Chemother. 2007 51(4): 1556–1558.

9. K. Ghosh, X.D. Ren, X.Z. Shu, G.D. Prestwich, R.F.A. Clark Fibronectin functional domains coupled to hyaluronan stimulate adult humandermal fibroblast responses critical for wound healing.Tissue Eng 2006; 12 : 601–613.

10. Wang JJ, Zeng ZW, Xiao RZ, Xie T, Zhou GL, Zhan XR, Wang SL. Recent advances of chitosan nanoparticles as drug carriers Int J Nanomedicine. 2011; 6: 765–774.

11. Wang Y, Tran KK, Shen H, Grainger DW. Selective local delivery of RANK siRNA to bone phagocytes using bone augmentation biomaterials Biomaterials. 2012 ;33:8540-8547.

12. Jebahi S, Oudadesse H, Abdessalem N, KeskesH, RebaiT, el FekiH, el Feki A. Comparative study of bone microarchitactural structure after porous bioglass and Strontium doped bioactive glass bone graft in Wistar rat model Journal of Scientific and Innovative Research 2014; 3 (1): 16-20.

13. W.J.E.M. Habraken, J.G.C. Wolke, A.G. Mikos, J.A. Jansen Injectable PLGA microsphere/calcium phosphate cements: physical properties and degradation characteristics J Biomater Sci Polym Ed 2006; 17: 1057–1074.

14. Peng L, Cheng X, Zhuo R, Lan J, Wang Y, Shi B, Li S. Novel gene-activated matrix with embedded chitosan/plasmid DNA nanoparticles encoding PDGF for periodontal tissue engineering. J Biomed Mater Res A. 2009; 90:564-576.

15. Guidoin R, Snyder R, Martin L, Botzko K, Marois M, Awad J, King M, Domurado D, Bedros M, Gosselin C. Albumin coating of a knitted polyester arterial prosthesis: An alternative to preclotting. Ann Thorac Surg 1984;37:457–465.

16. Kong X, Xu S, Wang X, Cui F, Yao J.Calcium carbonate microparticles used as a gene vector for delivering p53 gene into cancer cells. JBiomed Mater Res A. 2012 ;100:2312-2318.

17. Anderson, D. G.; Lynn, D. M.; Langer, R. Semi-automated synthesis and screening of a large library of degradable cationic polymers for gene delivery. Angew Chem Int Ed Engl 2003; 42: 3: 3153-3158.

18. Adler AF, Petersen LK, Wilson JH, Torres MP, Thorstenson JB, Gardner SW, Mallapragada SK, Wannemuehler MJ, Narasimhan B . Comb Chem High Throughput Screen. 2009;12:634.

19. An B, Desrochers TM, Qin G, Xia X, Thiagarajan G, Brodsky B, Kaplan DL. The influence of specific binding of collagen-silk chimeras to silk biomaterials on hMSC behavior Biomaterials ; 4,S0142-9612:01123-

20. Lacruz RS, Nakayama Y, Holcroft J, Nguyen V, Somogyi-Ganss E, Snead ML, White SN, Paine ML, Ganss BPLoS One. 2012; 7:e35200.

21. Chester J. Koh and Anthony Atala. Tissue Engineering, Stem Cells, and Cloning Opportunities for Regenerative Medicine. J Am Soc Nephrol 2004;15: 1113–1125.

22. Atala A, Kim W, Paige KT, Vacanti CA, Retik AB: Endoscopic treatment of vesicoureteral reflux with a chondrocyte-alginate suspension. J Urol 1994;152: 641–644.

23. Cai K, Zhang J, Deng L, Yang L, Hu Y, Chen C, Xue L, Wang L. Physical and biological properties of a novel hydrogel composite based on oxidized alginate, gelatin and tricalcium phosphate for bone tissue engineering. Adv Eng Mater 2007;9:1082–1088.

24. Song M, Wang X, Li J, Zhang R, Chen B, Fu D . Effect of surface chemistry modification of functional gold nanoparticles on the drug accumulation of cancer cells J Biomed Mater Res A 2008; 86:942-946.

25. Nandi S.K, S. Roy, P. Mukherjee, B. Kundu , D.K. De & D. Basu. Orthopaedic applications of bone graft & graft substitutes: a review. Indian J Med Res 2010;132: pp 15-30.

26. Santos MH, Valerio P, Goes AM, Leite MF, Heneine LG,Mansur HS. Biocompatibility evaluation of hydroxyapatite/collagen nanocomposites doped with Zn+2. Biomed Mater 2007; 2 : 135-141.

27. Chen,A.Z,Chen,M.Y,Wang,S.B, Huang,X.N,Liu,Y.G,andChen, Z. X.Poly(L-histidine)-chitosan/alginatecomplexmicro- capsule asanoveldrugdelivery agent. J.Appl.Polym.Sci. 2012; 124: 3728–3736.

28. Lammers T, Subr V, Ulbrich K, Peschke P, Huber P E, Hennink WE, Gert S, Kiessling F12, Adv Eng Mat 2010. B413–B421.

29. Yang Lei, Zhang L, Thomas J Webster. Anobiomaterials: State of the Art and Future Trends Adv Eng Mat 13, 2011, B197–B217.

30. Kadriye Tuzlakoglu and Rui L. Reis. Biodegradable Polymeric Fiber Structures in Tissue Engineering. tissue eng part b rev 2009, 15: 17-27.

31. Variola F, Brunski JB, Orsini G, Tambasco de Oliveira P, Wazen R, Nanci A. Nanoscale urface modifications of medically relevant metals: state-of-the art and perspectives Nanoscale. 2011 ;3:335-353.

32. Chao X, Zhang Z, Guo L, Zhu J, Peng M, Vermorken AJ, Van de Ven WJ, Chen C, Cui Y . A novel magnetic nanoparticle drug carrier for enhanced cancer chemotherapy PLoS One 2012;7: 40388.

33. Ghosh D, Lee Y, Thomas S, Kohli AG, Yun DS, Belcher AM, Kelly KA. l.M13-templated magnetic nanoparticles for targeted in vivo imaging of prostate cancer. Nat Nanotechno 2012 16;7: 677-682.

34. Yan G, Wang Y, He X, Wang K, Su J, Chen Z, Qing Z. A highly sensitive electrochemical assay for silver ion detection based on un-labeled C-rich ssDNA probe and controlled assembly of MWCNTs. Talanta 2012; 94:178-183.

35. Li Z, Kawamura K, Kawashita M, Kudo TA, Kanetaka H, Hiraoka M. In vitro assessment of poly(methylmethacrylate)-based bone cement containing magnetite nanoparticles for hyperthermia treatment of bone tumor. J Biomed Mater Res A. 2012 ;100:2537-2545.

36. Giovanna M, D. Di Lorenzo, N. Steimberg.Modelling tissues in 3D: the next future of pharmaco-toxicology and food research. Genes Nutr 2009; 4: 13–22.

37. Debeb *et al*. Characterizing cancer cells with cancer stem cell-like features in 293T human embryonic kidney cells. Molecular Cancer 2010, 9:180.

38. N Alno , F Jegoux , P P-M , S Tricot-Doleux ,H Oudadesse , G Cathelineau , G De Mello . Development of a three-dimensional model for rapid evaluation of bone substitutes in vitro: effect of the 45S5 bioglass, J Biomed Mater Res A 2010 ; 95:137-45. J Biome²d Mater Res A 2010 ; 95:137-145.

39. Van Kooten TG, Klein CL, Kirkpatrick CJ. Cell-cycle control in cell-biomate nteractions: expression of p53 and Ki67

in human umbilical vein endothelial cells in contact and extract testing of biomaterials. J Biomed Mater Res 2000 ; 52:199-209.

40. Mulrane L, Rexhepaj E, Penney S, Callanan JJ, Gallagher WM. Automated image analysis in histopathology: a valuable tool in medical diagnostics. Expert Rev. Mol. Diagn. 2008;8(6):707-725.

41. Goodpaster T, Legesse-Miller A, Hameed MR, Aisner SC, Randolph-Habecker J, Coller HAJ. An immunohistochemical method for identifying fibroblasts in formalin-fixed, paraffin-embedded tissue Histochem Cytochem. 2008 ;56:347-3458.

42. Kishida A, Kato S, Ohmura K, Sugimura K, Akashi M.Evaluation of biological responses to polymeric biomaterials by RT-PCR analysis. I. Study of IL-1 beta mRNA expression. Biomaterials 1996 13:1301-1305.

43. Chang DT, Jones JA, Meyerson H, Colton E, Kwon IK, Matsuda T, Anderson JMJ. Lymphocyte/macrophage interactions: biomaterial surface-dependent cytokine, chemokine, and matrix protein production .Biomed Mater Res A. 2008 1;87:676-687.

44. Xynos ID, Edgar AJ, Buttery LD, Hench LL, Polak JM.Gene-expression profiling of human osteoblasts following treatment with the ionic products of Bioglass 45S5 dissolution. J. Biomed. Mater. Res 2001; 55:151-157.

45. [132] Lü X, Lu H, Zhao L, Yang Y, Lu Z. Genome-widepathways analysis of nickel ion-induced differential genes expression in fibroblasts. Biomaterials 2010; 31:1965-1973

46. Han P, Ji WP, Zhao CL, Zhang XN, Jiang Y. Improved osteoblast proliferation, differentiation and mineralization on nanophase Ti6Al4V. Chin Med J (Engl) 2011; 124:273-279.