Study of Hydroxyapatite (HAp) Based Biocompatible Composite Matrix for Osseous Tissue Regeneration

[1]Abhineet Banerjee, [2]Sonjoy Roy Choudhury, [3]Sonjoy Roy Choudhury, [4]Dibyendu Debnath, [5]Biswadeep Chaudhuri*

[1-3,5]Department of Biotechnology, University of Engineering and Management, Action Area III, Kolkata, West Bengal 700156

[4]Scientist II, Aramco Performance Materials (APM) at Aramco, Rochester, New York

*Corresponding (chaudhuri.biswadeep@gmail.com).

Abstract

Tissue Engineering is emerging as a next generation therapeutical methods to overcome shortcomings of tissue defects from within using living cells and multidisciplinary fields of science and technology. Human beings have been greatly affected by various diseases which lead to degradation of bone tissue such as osteoporosis. It is difficult for the human body to regrow bone tissue affected by such diseases. For this reason it is necessary to develop methods by which bone tissue can be grown in vitro and can then replace the degraded tissue in human beings. Hydroxyapatite (HAp) being structurally similar to bone tissue of living organisms can be actively used as a matrix for the culturing of osteoblast cells which can then be incorporated into a human being at the diseased site to regenerate osseous tissue. Moreover HAp is widely used as a matrix due to its increased biocompatibility as well as its exceptional osteoconductivity. In addition, HAp has also been used in the form of composite like HAp-chondroitin sulphate(HAp-ChS) composite matrices that have been tested on Japanese white rabbits and have yielded significant results. Furthermore, metal ions doped with HAp have also been known to yield greater osteoconductivity. One of the most prominent of these metal ions is Zinc which can be found as a trace metal in bone and aids in bone metabolism and development. Zinc is known to initiate the activity of the enzyme aminoacyl-tRNAsynthetase which thus helps in translation and leads to protein synthesis. It also helps in the expression of genes which code transcription factors such as the Runx2 transcription factor required for differentiation of osteoblast cells as well as optimises the development of osteoclast cells to regulate resorption. Furthermore, zinc ions are capable of showing antimicrobial and antifungal properties which are extremely beneficial to prevent contamination during cell culture. MG63 osteoblast cells are primarily used for cell culture on HAp matrix generally for a few weeks to observe the efficiency of the matrix. MG63 cell lines have characteristics which are nearest to human osteoblast cells. Different matrices with same composition but varying surface roughness are also prepared to increase adhesion of the cells to the matrix, determined by various cell viability assays. It is found through comparative study that zinc doped HAp matrices yield significant growth of cells in comparison to pure HAp matrices when formed at a temperature of 1250oC and that does not exhibit any cytotoxic effect. In the present report an idea of the various research work conducted for the development of zinc doped Hap matrix in the field of bone tissue engineering is provided.

Keywords

Osteogenic Tissue Regeneration, Composite Material, Wound Healing, Bioimplants

1. Introduction

Osseous tissue is one of the most useable part of body that provides load bearing capability and supports movement. Bone provides load bearing support and protection to other organs required for the proper functioning of the human body. Bone is also the greatest source of Calcium and Phosphorous in the human body and acts as storage for these minerals. Bone disorders such as osteoporosis are very frequent all around the world and it is also predicted that about 40% women above the age of 50 will suffer from osteoporotic diseases. Data collected in the United States shows that such diseases are found in 124 out of every 1000 people [1]. Due to the increased occurrence of these disorders it is necessary to develop a method of replacing the damaged bone tissue. This has led to the development of Hydroxyapatite (HAp) matrices on which osteoblast cells can be cultured. HAp is a naturally formed mineral of Calcium apatite with the molecular formula $Ca_{10}(PO_4)_6(OH)_2$ and consists of a hexagonal crystal structure. Being similar to the structure of bone HAp acts as an efficient surface for the growth of osteoblast cells leading to the generation of osseous tissue.

HAp/Chondroitin sulphate(ChS) and HAp/hyaluronic acid(HyA) composite matrices offer increased biocompatibility as well as increased osteoconductivity when tested after implanting into the tibia and femur of Japanese white rabbits through injection.[2] The osteoconductivity is also known to be enhanced greatly when the HAp matrix is doped with metal ions. Of these metal ions Zinc (Zn) is considered one of the best to be doped with HAp matrices. Different HAp matrices with varied surface roughness can be used to determine effective adhesion of osteoblast cells. Zn is a trace element present in a significant amount in bones and is known to have stimulatory effect on osteoblast cell differentiation by the activation of gene expression of the transcription factor Runx2 which is involved in the differentiation of osteoblast cells. At the same time Zn has inhibitory influence on osteoclast cell activity as it promotes the apoptosis of mature osteoclast cells. Osteoporotic patients have been found to have reduced Zn amounts in their skeletal system [3]. Another importance of Zn is its involvement in as many as 300 enzymatic reactions taking place in the human body. It is also extremely important for development during prenatal and postnatal periods [4]. This ensures that the addition of Zn to the HAp matrix are a huge benefit for such patients. Furthermore, the HAp matrix is also protected from bacterial as well as fungal contamination due to the antimicrobial and antifungal activity of Zn.

Zinc in the form of Zinc Oxide(ZnO) shows antimicrobial property as its semiconductor properties allow it to produce Reactive Oxygen Species(ROS)[5] which targets DNA and causes oxidation of the nitrogen bases primarily Guanine. This causes mutation and thus causes damage to the bacterial DNA [6]. Zn can affect microbial membranes as ZnO particles or Zn2+ ions produced in aqueous medium directly comes in contact with it. Moreover, Zn2+ ion concentrations above the optimal level for microorganisms can disrupt cellular homeostasis and it may enter the prokaryotic cells thus becoming cytotoxic. By affecting the nucleic acids in the prokaryotic cell it may also inhibit the formation of certain enzymes required for the process of respiration [5,6].

The primary source of cells used to test the biocompatibility of Zn doped HAp matrix are the MG63 osteoblast cells. The efficiency of adhesion to the matrix and rate of proliferation of the cells have been studied for a maximum time of two weeks. Comparative studies have been performed using MG63 osteoblast cells where pure HAp matrix and Zn doped HAp matrices have been used in which Zn is sintered at temperatures of 1100OC and 1250OC [7]. HAp doped with different concentrations of Zn (1,2 and 5 mol%) to form Zn-HAp nanoparticles have also been used where Zinc nitrate has been used as the source for Zn.

Different concentrations of this nanoparticle has been tested in drug development for cancer treatment as well to test its efficiency in bone tissue engineering [4].

This review focuses on the summation of the various research work that has taken place in the field of bone tissue engineering primarily using Zn-HAp as well as HAp/ChS combinations to help in understanding the research work that have already been performed so that further novel ideas can be developed using this knowledge.

2.Various research work performed in this field

2.1. Bone and its components

Bone is the organ which gives support, acts as a defence for softer organs and provides a distinct structure to the body. The two major components of bone are calcium phosphate and collagen which makes it discernible from other similar structures such as chitin or enamel.Another important function of bones is that the bone marrow present in it is an important site for the production of red blood cells as well as white blood cells. Bone cells also known as osteocytes occupy a maximum of 15 percent of the volume of bone but in the case of mature bones of higher organisms it generally takes up about a mere 5 percent. The majority of bone is covered with non living material of which collagen is the protein presentbetween the osteocytes. The major minerals present in this region are calcium and phosphorus. The minerals exist in the form of apatite minerals of which the primary constituent is hydroxyapatite. The other two types of cells are the osteoblast and osteoclastcells. Osteoblast cells are those cells that result in the formation of bone cells by deposition on the matrix of the bone. Osteoclast cells are multinucleated cells whose main purpose is the resorption of bone tissue which takes place to meet the body's requirement of calciumin cases of calcium deficiency and also for restructuring the bone during stressful environmental conditions [8].

2.2 Disorders of the Bone

Disorders related to the bone are extremely common in human being and especially in elderly people. These disorders primarily weaken the bones and make them brittle. The most common of these bone diseases is osteoporosis [9].

2.2.1 Osteoporosis

Osteoporosis is primarily a disease where loss of osseous tissue takes place and gradually the bone becomes thinner. This disrupts the balance which maintains equilibrium between the formation of bones and the resorption of bones. Hence osteoporosis may arise due to hyperactivity of osteoclast cells and reduced activity of osteoblast cells. It is observed that osteoporosis is most prevalent in women who have undergone menopause [10].

3. HAp based platform

In order to combat such disorders of the bone, technology involving the in-vitro development of osteoblast cells on various biocompatible matrix came into existence. These cells could then be implanted into an individual at the affected site where the cells provided would replenish the lost cells. The most widely used matrix for the culture of osteoblast cells is the Hydroxyapatite(Hap) matrix which has the chemical formula Ca5(PO4)3(OH). Although HAp does not produce any toxic effect in the body and it also does not induce any immune response, it does however have a disadvantage. HAp does not have a significant mechanical strength and hence cannot provide sufficient support to the load bearing parts. For this reason it becomes necessary to add certain conjugates in order to improve the strength of the pure HAp. [11] Most conjugates are added in the form of polymers, chemicals such as chondroitin sulphate (ChS) as well as metal ions such as Zinc (Zn).

4. HAp based composites and metal ion doping

4.1 HAp/ChS conjugates

HAp conjugates are extensively being used for osseous tissue regeneration and coating purpose on various metallic bioimplants to accelerate cellular interaction. In this context, ChS is an important part of the extracellular matrix of connective tissues such as bone and cartilage. It can be described as a sulphated glycosaminoglycan with an extended unbranched polysaccharide chain consisting of a repeating disaccharide structure of N-acetylgalactosamine and glucuronic acid [12]. However various studies have been performed where HAp/ChS have been further hybridised with other chemicals. Studies involvingHAp/ChS/Chitosan(CS)/Hyaluronic acid(HA) have been performed on osteoblast cell cultures where cells have shown viability greater than 91% by performing MTT assay for an observation period of 7 days. This proves that this hybrid scaffold has greatly reduced toxic effects and is perfectly biocompatible [13]. Another important combination ofHAp/ChS is the addition of the metal oxide such as Titanium dioxide (TiO2). This combination has greater bioactivity and also presents antimicrobial properties. Titanium nanoparticles are also approved by the Food and Drug Administration (FDA) and hence are not responsible for producing toxic effect. Furthermore, toxicity studies have been performed using MG63 cell line and in vivo studies have also been conducted in Zebrafish (Danio rerio) embryos due to its genetic resemblance to human beings and also due to its increased generation in less time and reduced cost. These studies have indicated that there is no toxic effect on MG63 cell lines as well as no mortality was observed in the case of zebrafish [14].

4.2 Zn HAp combinations

Zn has been regarded as one of the best metal ions which can be added along with the HAp matrix as Zn is the second most plentiful trace element in the body and is mostly stored in bone and muscle with the amount in bones being 110-300 mg/Kg [4] Zn plays an important role in the production of the matrix of the collagen protein threads which act as the base for deposition of bone forming calcium and phosphorus. Another necessity of Zn is its role in the formation of enzymes required for degrading and reusing exhausted bone proteins [15,16]. The combination of Zn-HAp has been used greatly in studies of efficiency of drug delivery keeping ciprofloxacin as the model drug. The antibacterial property of Zn is also another important reason because of which Zn-HAp has become important in recent studies. Zn ion shows inhibitory effect on the activities of living bacterial cells such as the energy producing process of glycolysis as well as the synthesis of polysaccharides required for the cell wall. Zn-HApnanorods have been created to test their antibacterial property on oral bacteria such as Aggregati bacteria ctinomycetemcomitans, Streptococcus mutans, and Fusobacterium nucleatum. Zn can also be used in its oxide form as ZnO nanoparticles (NP) can be formed to combine with the HAp matrix. ZnO NPs are non toxic as well as completely biocompatible and can resist bacteria by displacing magnesium ions in bacterial cells and thus disrupting their enzymatic reactions to cause bactericidal effects [4,16]. Furthermore, ZnO NPs have also been synthesised biologically in ways different from its chemical origins. Sargassummuticum , a brown marine algae, extracts have been studied to produce ZnO NPs by utilising the polysaccharides present in it through the fast and efficient process of green synthesis [17].

Conclusion

Thus it can be understood from this review that the use of HAp matrix along with conjugates of ChS and Zn provides a substantial contribution in the field of bone tissue regeneration.

Many more such combinations can be made by utilising other metal ions as well as other chemical compounds all in the hope of arriving at a novel creation which can be considered nearly ideal for the efficient healing of bone tissue and perhaps even a permanent cure to preventing further damage of bones by various bone disorders or by physical injury.
Further research must also be performed regarding the human body to reveal certain chemicals which play an important role in bones but is unknown. The knowledge of such chemicals can further provide improved methods of osseous tissue regeneration for the betterment of all human beings.

Acknowledgement

Authors are grateful to UEM Kolkata for various supports for this extensive review work.

References

1. Lyckfeldt O, Ferreira JM. Processing of porous ceramics by starch consolidation. J Eur Ceram Soc, 18:131–40.1998.
2. Toohey KS, Sottos NR, Lewis JA, Moore JS, White SR. Self-healing materials with microvascular networks. Nat Mater.7:56–63.2007.
3. Rivera-Muñoz E, Velázquez R, Rodríguez R. Improvement in mechanical properties of hydroxiapatite objects with controlled porosity made by modified gelcasting process. Mater Sci, 426:4489–94.2003.
4. Zhao CaiXia, Zhang WeiDe. Preparation of waterborne polyurethane nanocomposites: Polymerization from functionalized hydroxyapatite. Eur Polym J,44: 1988–95.2008
5. Tassel X, Barbry D, Tighzert L. A new blocking agent of isocyanates. Eur Polym J ,36:1745–51.2000.
6. Mamunya YP, Shtompel VI, Lebeder EV, Kanapitsas PA, Boiteux G. Structure and water sorption of polyurethane nanocomposites based on organic and inorganic components. Eur Polym J, 40:2323–31.2004.
7. Talukdar Y, Raslkow JT, Lalwani G, Kanakia S. Biomaterials, 35:4814–77,2014.
8. Chung C, Kim YK, Shin D, Ryoo SR, Hong B, Min D. Acc Chem Res, 46:2211–24.2013
9. Sebaa M, Nguyen TY, Paul RK, Mulchandrani A, Liu H. Mater Lett,92:122–5. 2013
10. Choi JC, Lee SJ, Christ JG, Atala A, Yoo JJ. Biomaterials,29:2899–906,2008
11. Bhadra D, Sanigrahi J, Chaudhuri BK, Sakata H. Polym Compos,33:436–41.2012
12. Chaudhuri B, Pramanik K. Adv Sci Eng Med,5:427–30. 2013
13. Chen W, Yan l, Bangal PR. Carbon,48:1146–52. 2010

14. Dikin DA, Stankovich S, Zimney EJ, Piner RD, Dommett HB, et al. Nature, 448:457–60,2007

15. Mei Y, Wang Z, Li XJ. Appl Polym Sci ,129:1089–94.2013

16.Madhavi S, Ferraris C, White TJ. Synthesis and crystallization of macroporous hydroxyapatite. J Solid State Chem 2005;178:2838–45.

17. Prado da Silva MH, Lemos AF, Gibson IR, Ferreira JMF, Santos JD. Porous glass reinforced hydroxyapatite materials produced with different organic additives. J Non-Crystall Solids 2002;304:286–92.