International Journal of Engineering & Scientific Research
Vol. 6 Issue 1, January 2018, (Special Issue ICON17-MCC)
ISSN: 2347-6532 Impact Factor: 6.660
Journal Homepage: http://www.esrjournal.com Email: esrjeditor@gmail.com
Double-Blind Peer Reviewed Refereed Open Access International Journal - Included in the International Serial Directories Indexed & Listed at: Ulrich's Periodicals Directory ©, U.S.A., Open J-Gage as well as in Cabell's Directories of Publishing Opportunities, U.S.A

Effect of Multiwalled Carbon nanotube Zinc oxide composite scaffold for the growth of nano Hydroyapatite for bone regeneration.

Dr. Nasera Rizwana *
N.Pradeep *
V.Chaitra *
Uma.V **

Keywords:
Carbon nanotube,
ZincOxide,
Nanocrystalline hydroxyapatite,
Scaffold,
Simulated Body Fluid,
Osteoinductive,
Osteoconductive,
Composites

Abstract

Bone defects are major issue for the clinician to cure and much effort or skill to accomplish. Bone defects can occur due to trauma, infection and other diseases like osteoporosis or cancer. Hydoxyapatite scaffolds have been long used in graft procedures, hip replacements, dental implants and to repair early lesions in tooth enamel. But their properties like tensile strength and fracture toughness is not similar to bone. To overcome this bone tissue engineering has become a good alternative. Provide suitable environment of cell growth and help in release of growth factors. Hydroxyapatite (HAp) is a wonderful biocompatible material to support tissue adhesion and bone growth. Carbon nanotubes are considered as both osteoinductive and osteoconductive material and have high aspect ratio and good mechanical properties which strengthen hydroxyapatite. Furthermore zinc oxide is also known to form bone like apatite layer and have good mechanical and biological

***Department of Nanoscience and Technology, Mount Carmel College, Bengaluru-52**

****Department of Electronics, Mount Carmel College, Bengaluru-52**

properties. In this report we grown the nanocrystalline hydroxyapatite (nHAp), (it has characteristics of natural bone) by chemical technique with support of multiwalled carbon nanotubes and multiwalled carbon nanotubes, zinc oxide composites. The effects of zinc oxide on CNT

Bone defects are major issue for the clinician to cure and much effort or skill to accomplish. Bone defects can occur due to trauma, infection and other diseases like osteoporosis or cancer. Hydoxyapatite scaffolds have been long used in graft procedures, hip replacements, dental implants and to repair early lesions in tooth enamel. But their properties like tensile strength and fracture toughness is not similar to bone. To overcome this bone tissue engineering has become a good alternative. Provide suitable environment of cell growth and help in release of growth factors. Hydroxyapatite (HAp) is a wonderful biocompatible material to support tissue adhesion and bone growth. Carbon nanotubes are considered as both osteoinductive and osteoconductive material and have high aspect ratio and good mechanical properties which strengthen hydroxyapatite. Furthermore zinc oxide is also known to form bone like apatite layer and have good mechanical and biological properties. In this report we grown the nanocrystalline hydroxyapatite (nHAp), (it has characteristics of natural bone) by chemical technique with support of multiwalled carbon nanotubes and multiwalled carbon nanotubes, zinc oxide composites. The effects of zinc oxide on CNT

Bone defects are major issue for the clinician to cure and much effort or skill to accomplish. Bone defects can occur due to trauma, infection and other diseases like osteoporosis or cancer. Hydoxyapatite scaffolds have been long used in graft procedures, hip replacements, dental implants and to repair early lesions in tooth

enamel. But their properties like tensile strength and fracture toughness is not similar to bone. To overcome this bone tissue engineering has become a good alternative. Provide suitable environment of cell growth and help in release of growth factors. Hydroxyapatite (HAp) is a wonderful biocompatible material to support tissue adhesion and bone growth. Carbon nanotubes are considered as both osteoinductive and osteoconductive material and have high aspect ratio and good mechanical properties which strengthen hydroxyapatite. Furthermore zinc oxide is also known to form bone like apatite layer and have good mechanical and biological properties. In this report we grown the nanocrystalline hydroxyapatite (nHAp), (it has characteristics of natural bone) by chemical technique with support of multiwalled carbon nanotubes and multiwalled carbon nanotubes, zinc oxide composites. The effects of zinc oxide on CNT- nHAp has studied. The prepared CNT, ZnO CNT composite kept in Simulated Body Fluid (SBF) for about 4 weeks as a scaffold. The sample was characterized after growth of four weeks. The SEM image reveals the growth of nanocrystalline hydroxyapatite more in zinc oxide CNT composites the CNT. EDAX confirms the prepared material is hydroxyapatite. The growth mechanism was discussed.

Author correspondence:

Dr .Nasera Rizwana
Masters program, Department of Nanoscience and Technology
Mount Carmel College, Vasanthnagar, Bengaluru, 560052, Karnataka, India.

1. Introduction

Bone defects can occur due to trauma, infection and other diseases like osteoporosis or cancer. These bone defects are usually treated by bone grafts like allografts and autograft

[1]. The tissue regeneration capacity of these grafts is measured in terms of their osteoconductive and osteoinductive potential [2].Osteoconductive potential refers to the property of scaffold or a graft that causes cells to grow. Osteoinductive potential is that property of scaffold which causes stimulation of Mesenvhymal stem cells (MSCs) to differentiate into preosteoblasts to begin the bone forming process [3].But the disadvantage of using these grafts is that they may cause post operative complications like pain, infection, scarring, blood loss and immune rejection. These disadvantages of the grafts has led to the use of scaffolding and tissue engineering to treat bone defects.

Nanoscaffolding is the process in which a 3-D structure of nanometer scale composed of materials on which bone or tissues are grown [4]. These scaffolds when placed inside the body provide a base for the cells to regenerate. The scaffolds should be rigid, biodegradable, biocompatible, should promote adhesion and proliferation of cells and have the ability to be penetrated by blood vessels and body fluid [5].

Hydroxyapatite (HA), $Ca_{10}(OH)_2(PO_4)_6$ is a bio ceramic mineral which is chemically similar to the bone. HA has been found to be similar to the inorganic materials found in bone. It has both osteoconductive and osteoinductive property. As it is similar to bone, it is biocompatible and also biodegradable. It has good interaction with soft and hard tissues [6]. It is porous material which has the ability to allow the in growth of blood vessels. Even though it has good properties, it also has a disadvantage that is it has low mechanical strength and hence it should be used together with other materials.

Carbon nano tube (CNTs) are unique nano materials which have good mechanical, chemical, and electrical properties. So when CNTs are combined with HA, they provide better mechanical strength. CNTs have high tensile strength and are good substrate for cell growth and differentiation [7].CNT and nHAp composite is also known to have enhanced strength, fracture toughness and favorable osteoblast cell proliferation properties

[8].Additionally, it is also known that Zinc oxide (ZnO) helps in formation of bone like apatite layer and also has good mechanical and biological properties [9].

In this study, we have grown nHAp on CNT alone and on Zno CNT composite. The prepared composites were placed in Simulated Body Fluid (SBF) for 4weeks as a scaffold. The prepared sample was characterized.

2. Research Method

2.1 Materials

a. Multi walled Carbon Nanotubes

b. Chemical reagents for preparing nHAp-CNT and nHAp-CNT-ZnO composite : $CaCO_3$, H_3PO_4, NH_4OH, concentrated ammonia solution, ZnO.

c. For Simulated body fluids NaCl, $NaHCO_3$, KCl, $K_2HPO_4.3H_2O$, $MgCl_2.6H_2O$, $CaCl_2$, $NaSO_4$,HCl and TRIS

2.2 Synthesis of nHAp and CNT composite scaffold

MWCNT were prepared using chemical vapor deposition. 15mg MWCNT was added to 100 ml of con. NH_4OH and bath sonicated for 30min to obtain homogeneous solution. nHAp was prepared by wet chemical precipitation method [8]. 2 gm of $CaCO_3$ was dissolved under magnetic stirring in 100ml of 0.3M H_3PO_4. The resulting solution was filtered using Whattmann filter paper and a colorless dihydrogen phosphate precursor solution was obtained. To the CNT solution, the precursor solution was added drop wise under magnetic stirring at room temperature keeping pH above 10 for 30min. The solution was filtered and washed with distilled water.the solid obtained was dried under vaccum overnight.

2.3 Synthesis of nHAp, CNT, ZnO composite scaffold

15mg CNT was added to 100 ml of con. NH_4OH and bath sonicated for 30min to obtain homogeneous solution. 2gm of ZnO and 2 gm of $CaCO_3$ was dissolved under magnetic stirring in 100ml of 0.3M H_3PO_4. The resulting solution was filtered using Whattmann filter paper and a colorless dihydrogen phosphate precursor solution was obtained. To the CNT solution, the precursor solution was added drop wise under magnetic stirring at room temperature keeping pH above 10 for 30min. The solution was filtered and washed with distilled water.the solid obtained was dried under vaccum overnight.

2.4 Preparation of Simulated BodyFluid (SBF)

SBF is and inorganic physiologic solution having similar composition as that of human blood plasma. SBF was prepared according to ISO Norm 23317 [10].

2.5 Preparation of samples

10mg of nHAp,CNT composite and nHAp,CNT,ZnO composite samples were placed into a beaker containing 10ml of SBF. The samples were kept for 4weeks in SBF at 37^0C. The SBF solution was added every 24hrs. The samples were filtered, rinsed with distilled water and dried under vaccum overnight.

3. Results and Analysis

Scanning Electron Microscopy analysis(SEM) and Energy Dispersive Spectroscopy Analysis (EDAX) analysis was done for both the samples.

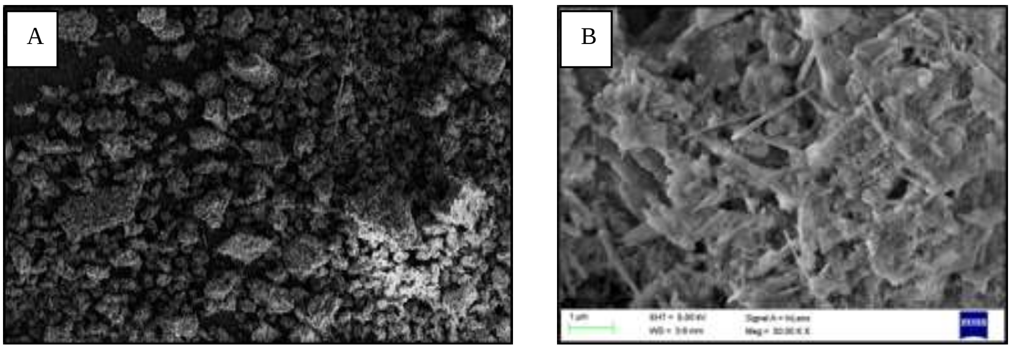


Fig 1. nHAp and CNT sample before placing in SBF (A). nHap growth on CNT after placing in SBF for 4 weeks.

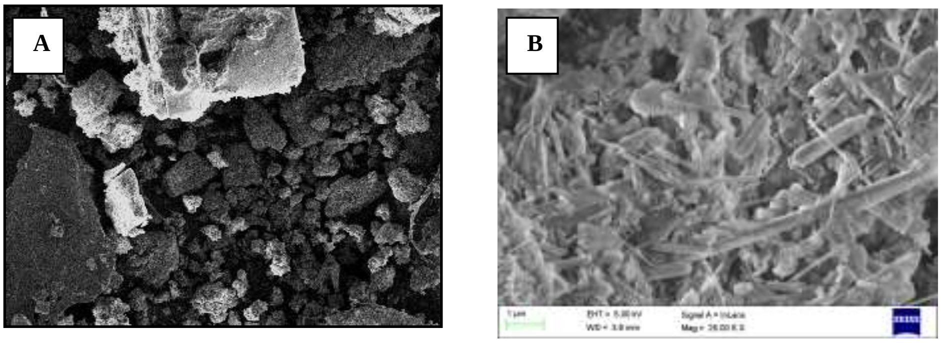
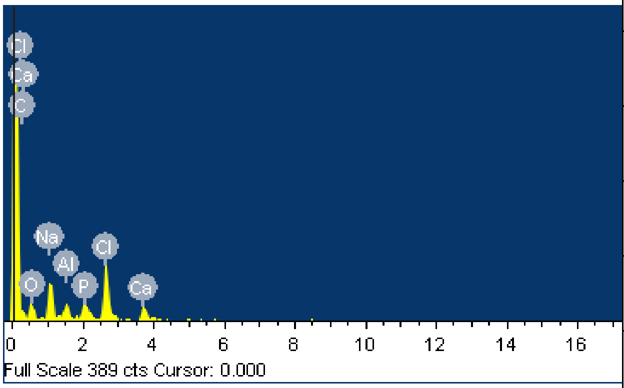


Fig 2. nHAp ,ZnO and CNT sample before placing in SBF (A). nHAp growth on CNT and ZnO scaffold after placing in SBF for 4 weeks.

In Fig 1(A) nHAp and MWCNT composite scaffold sample is seen before keeping it in Simulated Body Fluid. The SEM picture shows grain particles of the sample. Fig 1(B) shows the presence of MWCNT rods on which nHAp growth has occurred.

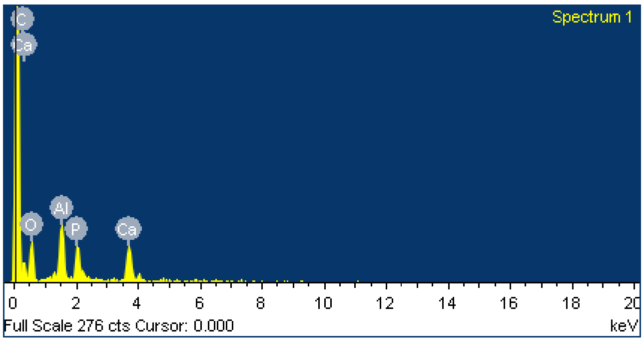
Fig 2(A) shows the nHAp ,MWCNT and ZnO sample before keeping it in Simulated Body Fluid. The SEM picture shows grain particles of different sizes of the sample. Fig 2(B) shows the presence of MWCNT rods on which nHAp growth has occurred. The growth on this sample is seen to be more than the nHAp-MWCNT composite scaffold.

Energy Dispersive Spectroscopy Analysis (EDAX)



ELEMENT	WEIGHT%	ATOMIC%
C K	30.85	46.46
O K	23.08	26.10
Na K	12.77	10.05
P K	4.02	2.35
Cl K	18.90	9.64
Ca K	7.14	3.22
TOTAL	100	100

Fig 3. EDAX Analysis of nHAp and CNT composite after placing in SBF for 4 weeks



ELEMENT	WEIGHT %	ATOMIC %
C K	28.40	40.66
O K	39.22	42.16
Al K	11.03	7.03
P K	7.89	4.38
Ca K	13.46	5.78
TOTAL	100	100

Fig 4. EDAX Analysis of nHAp grown on CNT and ZnO composite after placing in SBF for 4 weeks.

The EDAX analysis shows the weight% and atomic% of the elements present in both the scaffold samples. In nHAp-MWCNT composite scaffold (Fig 3) the weight% of Ca is 7.14% and that of phosphorus is 4.02%. And in nHAp-MWCNT-ZnO composite scaffold the weight% of Ca is 13.46% and that of phosphorus is 7.89% (Fig 4).

It is thus observed that there is increase in the amount of Calcium and Phosphorus in the scaffold containing Zinc oxide. However the presence of Zinc was not found in the area that was investigated.

4. Conclusion

The two composite scaffolds that is cnt and nHAp composite and CNT, ZnO and nHAp composite scaffold were prepared by wet chemical synthesis method. The SEM ananlysis shows growth of nHAp on both the scaffolds when placed in SBF for 4 weeks. The EDAX analysis of both the composite scaffolds confirms the presence of increased Calcium content that is growth of nHAp. But the presence of zinc was not found in the CNT,ZnO nHAp composite scaffold in the area that was investigated. Hence it is suggested to increase the amount of ZnO used in sample preparation. Furthermore the biocompatibility and toxic effects of the Zinc oxide should also be investigated.

References

[1]. Brydone A,Meek D, Maclaine S. 2010 " Bone grafting, orthopaedic biomaterials, and the clinical need of bone engineering."Process Institute of Mech Eng Part H December 2010 DOI: 10.1243/09544119JEIM770 [pub med]

[2]. Albrektsson T, Johansson C 2001 " Osteoinduction, osteoconduction and osseointegration." European Spine Journal 2001 S96-101. http://dx.doi.org/10.1007/s005860100282

[3]. Liliana.P, Magada.L, Jamie.R 2014 "Scaffold design for bone regeneration." Journal of Nanoscience and Nanotechnology 2014 DOI: 10.1166/jnn.2014.9127 · Source: PubMed

[4]. http://nanoscaffoldtech.com/may17,2013

[5]. Hisao.H et al 2012 "Basic potential of CNT in tissue engineering applications." Journal of nano materials Volume 2012 (2012), Article ID 343747, 10 pages http://dx.doi.org/10.1155/2012/343747

[6]. Michel, J., Penna, M., Kochen, J., & Cheung, H. (2015)." Recent Advances in Hydroxyapatite Scaffolds Containing Mesenchymal Stem Cells." Stem Cells International, *2015*, [305217]. DOI: 10.1155/2015/305217

[7]. Gupta et al 2015 "Biocompatibility of SWCNT composite for bone regeneration." Bone Joint Res. 2015 May; 4(5): 70–77. Published online 2015 May 1. doi: 10.1302/2046-3758.45.2000382

[8]. J.David et al 2014 "Integration and bioactivity of hydroxyapatite grown on carbon nano tubes and grapheme oxide." Carbon 2014 https://doi.org/10.1016/j.carbon.2014.08.020

[9]. Vojislav et al 2010 "Synthesis, characterization and antimicrobial activity of Copper and Zinc doped HA nano particles". Applied surface science Aug 2010 Vol 256 Issue 20 https://doi.org/10.1016/j.apsusc.2010.03.124

]10]. T. Kokubo, H. Kim, and M. Kawashita, 2003 "Novel bioactive materials with different mechanical properties," Biomaterials, vol. 24, no. 13, pp. 2161–2175, 2003.