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Chapter

Carbon Nanotubes Integrated Hydroxyapatite Nano-Composite for Orthopaedic and Tissue Engineering Applications

Khalid Parwez, Arun A. Bhagwath, Asif Zawed, Bhagwan Rekadwad and Suman V. Budihal

Abstract

The reassessment of the literature stipulates that an increasing amount of research in exploring the Hydroxyapatite Carbon Nanotubes (HA-CNT) system for orthopedic application. Chemical precipitation, CNT functionalization, and spray drying are the routinely used methods for CNT dispersal in HA matrix for the application such as bone tissue engineering, nanostructured scaffolds, dental regeneration, myocardial regeneration, and skin regeneration. Although mechanical strength and biocompatibility is a substantial concern for the fabrication of structures. Developing composite and bioceramic scaffolding with different natural and synthetic biomaterials are the futuristic approach in the biomedical engineering field. The problems such as biocompatibility, biodegradability, and mechanical resistance can be solved by combining natural, and artificial biomaterials. The natural biomaterials, such as collagen, cellulose, chitosan, have a close resemblance to the natural extracellular matrix (ECM). These materials are biocompatible, biodegradable. The artificial biomaterials, such as Poly Vinyl Pyrrolidone (PVP), Poly Capro Lactone (PCL), Poly Ethylene Glycol (PEG), and Poly Lactic Acid (PLA) are also the material of choice for the fabrication of the composite materials. Additional effort is necessary to fabricate biocompatible composite scaffolding for tissue engineering. Moreover, vascularization, differentiation, cellular proliferation, and cells to scaffold interaction are the foremost challenges in the area of tissue engineering that remains to overcome.

Keywords: Nanocomposites, Carbon Nanomaterial, Fabrication, Electrospinning, Bone Tissue Engineering

1. Introduction

The bone defects are generally caused by trauma, tumor resection, deformity, and infections. This kind of large bone defect has increased in recent years. Lack of resources for bone grafting as well as rejections of the grafts are potential obstacles in the treatment of these defects. Tissue engineering is considered the emerging viable technology for reconstructing tissues and organs structure. Tissue engineering is also helpful in repair, and reinforce the functions of dented tissues.

The electrospinning techniques have been widely used for fabricating ultrafine, and unremitting nano-fibres. The bone and dentin are natural mineralized hard tissues with extremely compound and classified structures [1–3]. The3-dimensional structures to the hard mineralized tissue are produced by the deposition of minerals to the structured organic matrix [4]. The primary minerals of the tissues are hydroxyapatite (HAp) (Ca10(PO4)6(OH)2) found in bone and dentine [5, 6].

To fabricate the bone and dentin like biomaterials, it is essential that the scientist from bioengineering, biomedical, biology, chemistry, and materials science should come together. The multidisciplinary approach of fabrication shall create biocompatible hard tissue with improved mechanical properties. The large aspect ratio of nanomaterials has attracted the extensive attention of material scientists across the globe. The large aspect ratio enables the nanomaterials to have a large surface area in less volume.

The following points shall be considered for the development of biomaterials for hard tissue regeneration.

i. The material shall be compatible with the regenerated tissue.

ii. The time for tissue regeneration and healing shall be reduced.

iii. There shall not be any inflammatory or toxic response to the host because of the materials used.

iv. The mechanical properties of material shall be equivalent to the natural bone or dentine material.

Recently, the biomimetic approaches to synthesize the natural composite materials are being discussed by the scientific communities [5]. The biomimetic approach of fabrication enables the nanomaterials to spread to a specific orientation in the matrix. Hence, it is expected that the newly synthesize composite shall have the morphology and composition of the natural tissues. The PVA is a water-soluble synthetic polymer. The PVA backbone is exceedingly interconnected by H-bond. The presence of the abundant amount of hydroxyl groups (–OH) in PVA polymer enables them to have better mechanical, chemical, and biological properties e.g., better tensile strength, excellent chemical resistance, biocompatibility, and stealth resistance. Moreover, the –OH group also allows appending the biological molecules like antibodies, DNA collagens, and hyaluronidase to PVA without any compatibility issues [6, 7]. The biocompatibility and biodegradability of PVA polymer to living tissues results in a wide range of biomedical and biopharmaceutical applications [8–10]. Two reasons to select PVA/HA nanocomposites for this study are (a) PVA and HA both has abundant hydroxyl groups (–OH), so, PVA can strongly interact with HA and, (b) aqueous phase electrospinning of PVA/HA nanocomposite is possible.

We propose an alternative approach to PVA-HA nanocomposite manufacturing in this research, which shall have a promising future application related to hard-tissue engineering. The electrospun PVA-HA composite shall be an alternative to bone replacement and dentine coating. Because, the electrospun composite shall have similar physicochemical properties, and resemble the nanostructure of living bone.

The synthetic hydroxyapatite is biocompatible, bioactive, and osteoconductive material [11], which is similar to the hydroxyapatite present in the living system. The chemical composition and molecular structures of the synthetic hydroxyapatite are similar to the minerals present in the dentine, cartilage, and bone. Thus, the

synthetic HA has been attracting the attention of the scientist in biomedical an application such as coating for the implant, prosthesis, and replacement of defective bone [12].

Hydroxyapatite is the integral component of natural bone, which is bioactive, biocompatible, and osteoconductive. Thus, this material is most widely used in tissue engineering. The brittleness and low biodegradability are considered as the limiting factor for this material [13, 14]. Hence, making the composite with PVA can enhance mechanical strength and biodegradability. The PVA/HA nanocomposite shall have a suitable pore size, good aspect ratio, and biocompatibility to the living cells [15].

Carbon nanotubes (CNTs) are classified as single-walled carbon nanotubes (SWNTs) and multi-walled carbon nanotubes (MWNTs). The CNTs are considered as a suitable nanomaterial for biomedical application because of their aspect ratio, excellent mechanical, thermal, and electromagnetic properties [16]. CNTs are proved to be biocompatible and promote attachments and proliferation of osteoblast cells. Hence, it can be assumed that the presence of CNTs in the matrix shall result in better and improved composite fabrication.

The electrospinning is a technology introduced in the year 1934. This technology is successful in producing nanofibers at a very low cost [17]. The cost-effectiveness and the dimension of produced fibers enable this technology to be used in the field of tissue engineering [18–20]. The as-produced structure of HA by electrospinning resembles natural ECM, which enables them to be used as a scaffold for tissue regeneration, immobilizing enzymes, wound dressing materials, and tissue culture [21–24]. In the present study, we synthesized composite fibers using PVA and HA via electrospinning and characterized by UTM, SEM analysis. The electro-spun three-dimensional structure is microscopically similar to bone.

2. Review of literature

Miniaturization of the devices encouraged the scientist to explore a polymer nanofiber. The electrospinning techniques enable them to produce controlled fiber diameter with high strength, which couldn't be achieved by the conventional fiber processing techniques.

The microstructure formed during electrospinning leads to the fabrication of strong fibers by systematic arrangements. It is important to understand the effect of electrospinning parameters on morphology, tensile strength, and fiber diameter. The electrospinning parameters affect the inherent structure and overall deformation behavior. The deformation behavior, tensile strength, and fiber size are mostly affected by crystallinity and molecular orientation. Whereas, the electro wiring affects the fiber diameters during the process of electrospinning [25–29]. Furthermore, the electric field applied during the electrospinning encourages the self-assembly of the fillers, which leads to composite synthesis in spatial arrangements. The CNTs and carbon black (CB) are the most commonly used fillers in the electrospinning process. These fillers disperse themselves within the fiber and provide high strength and toughness to the composite. This simulating behavior of the fillers make them a good candidate for the application such as tissue engineering, filtration, and advance nanofibers [30–34].

The characterization of the nanofibers for the traction behavior is the challenging part of the field because the nanofibers get deformed with low load. Consequently, the mechanical integrity of the nanofibers is less studied and understood. The characterization of the mechanical deformation of the nonwoven fabrics prepared by electrospinning is the need of the time. There are several parameters that affect the tensile properties of the nonwoven fibers, such as,

entanglement, orientation, porosity, fiber-fiber interaction, and size distribution of the fibers. Although, these parameters are not readily controllable in the nonwoven fibers, hence, characterization of the tensile properties of the single fibers is of very much interest. In recent studies, it has been shown that the cutting effect and size effects are important to control the intrinsic structure and diameter respectively [35–37].

3. Materials and Methods

3.1 Materials

- PVA (Polyvinyl chloride).
- HA (Hydroxy apatite).
- CNT (Carbon nanotubes).
- 0.5 M, 5 ml of Calcium Nitrate [Ca(NO3)2 4H2O].
- 0.5 M, 10 ml of Diammonium hydrogen phosphate [(NH4)2 HPO4].
- Ethanol.
- Distilled water.
- Magnetic stirrer.
- Cotton.
- Beakers.

3.2 Apparatus required

- Electrospinning Machine.
- Sonicator.
- Stirrer machine.
- UTM (Universal testing machine).
- SEM (Scanning electron microscopy).

4. Methods

4.1 PVA film formations

- 18 gm of PVA is taken in a beaker and It is mixed with 182 ml of distilled water.
- 200 ml of PVA solution is continuously stirred for 3 hrs using magnetic stirrer.

- Then this solution is loaded in a syringe upto 2 ml and fixed in the syringe clamp.
- Then either the drum collector or the square collector is connected so that the film is formed on it.
- Few parameters are set before starting the machine which are as followed:

  i. Voltage - 12Kv.

  ii. Flow-rate – 0.2 ml/hr.

  iii. Syringe volume -2 ml.

  iv. Needle diameter – 0.5 mm.

  v. Needle length -25 mm.

  vi. Distance between collector & syringe -18 cm.

  vii. Time of run – 8 hrs.

4.2 Formation of HA–PVA film

- 15 ml of PVA is taken in a beaker.
- 0.59gm of Ca(NO3)2 4H2O is added to 5 ml of distilled water.
- 0.57gm of (NH4)2HPO4 is added to 10 ml of distilled water.
- pH should be adjusted more than 10.
- The solution is stirred and ammonia solution is added into it.
- After preparing the solution it is loaded into the syringe upto 2 ml and make sure there won't be any bubble formation in the syringe.
- Then the syringe is adjusted to the suitable position.
- After that few parameters are set in order to form the film which are as follows:

  i. Voltage – 19Kv.

  ii. Flow - rate – 0.2 ml/hr.

  iii. Syringe volume – 2 ml.

  iv. Needle diameter – 1.2 mm.

  v. Needle length –38 mm.

  vi. Distance between the collector & syringe –

vii. Time of run – 8 hrs.

viii. Drum collector speed – 2000 rpm.

4.3 Formation of PVA-CNT film

- 15 ml of PVA is taken in a beaker and 1% of CNT (0.15gm) and sonicate it.
- Prepare two solutions of same quantity and sonicate them for 3 hrs.
- After that syringe is loaded with the solution which is prepared upto 2 ml.
- Then it is adjusted appropriately.
- Drum collector is connected on which the film will be formed.
- Few parameters are adjusted before starting the machine which are as follows:

i. Voltage – 19Kv.

ii. Flow-rate – 0.2 ml/hr.

iii. Syringe volume – 2 ml.

iv. Needle diameter – 1.2 mm.

v. Drum speed – 2000 rpm.

5. Characterization

5.1 Surface topography characterization

The surface morphology and atomic structure of PVA, PVA/HA, PVA/CNT (**Figure 1**) scaffolds were examined under the FESEM (Hitachi SU6600, Japan) and EDS (Horiba-EMAX). Prior to the microstructure analysis, specimens were coated with gold using an ion sputter coating instrument (Hitachi E – 1010, Japan) with a current set at a 15 mA for a coating time of 15 s.

5.2 Universal testing machine (UTM) analysis

A universal testing machine (UTM), also known as a universal tester materials testing machine or materials test frame, is used to test the tensile stress and compressive strength of materials. It is named after the fact that it can perform many standard tensile and compression test on materials, components and structures.

6. Results and discussion

6.1 Scanning electron microscope (SEM)

SEM micrographs of the samples were taken using (Zeiss) electron microscope, Japan. The samples were coated with gold using sputtering technique. Scanning

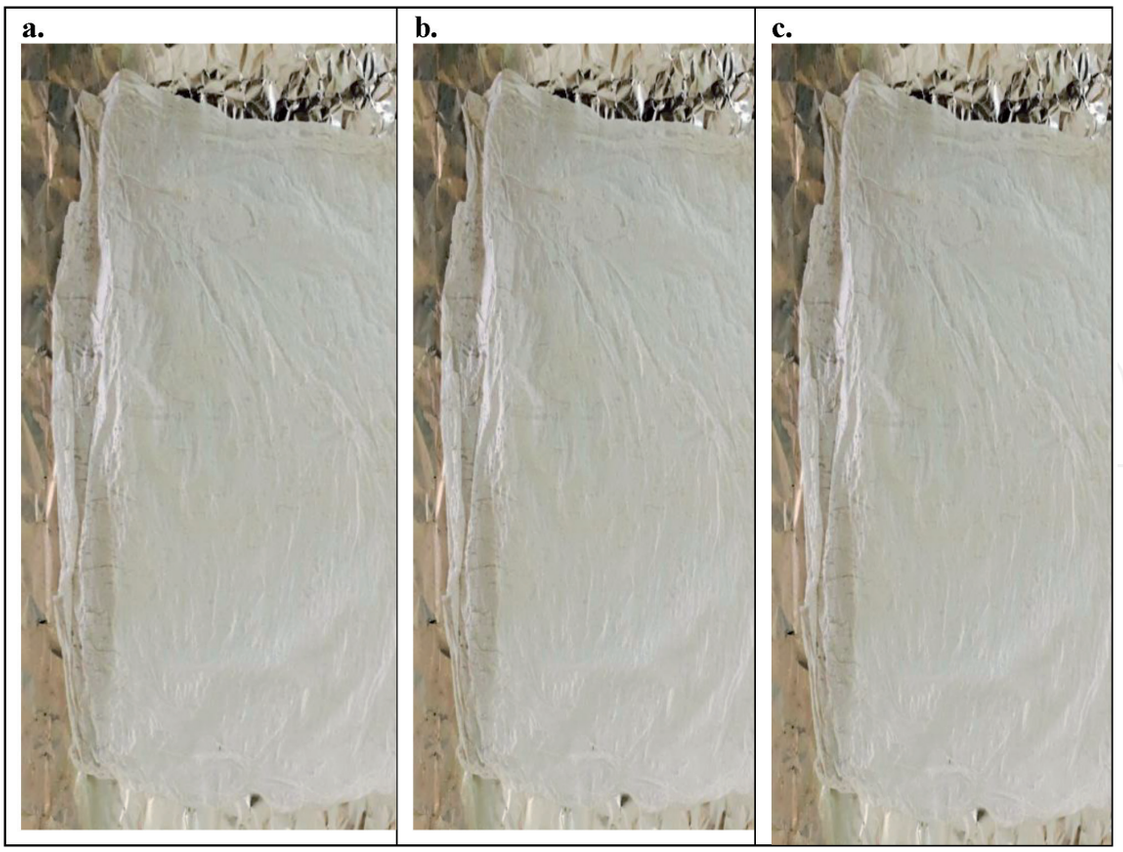


Figure 1.
a. PVA Film, b. PVA-HA Film, c. PVA-CNT Film prepared through Electrospinning.

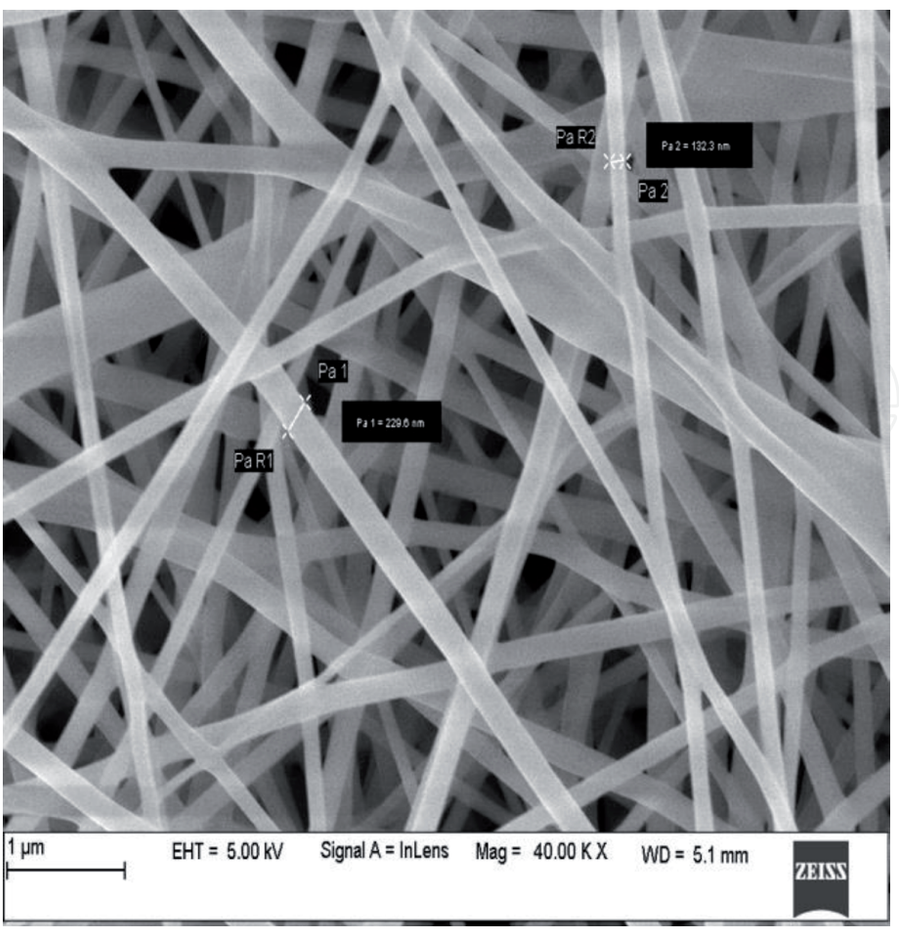


Figure 2.
SEM image of the PVA film, scale 1 µm and 4K X magnification.

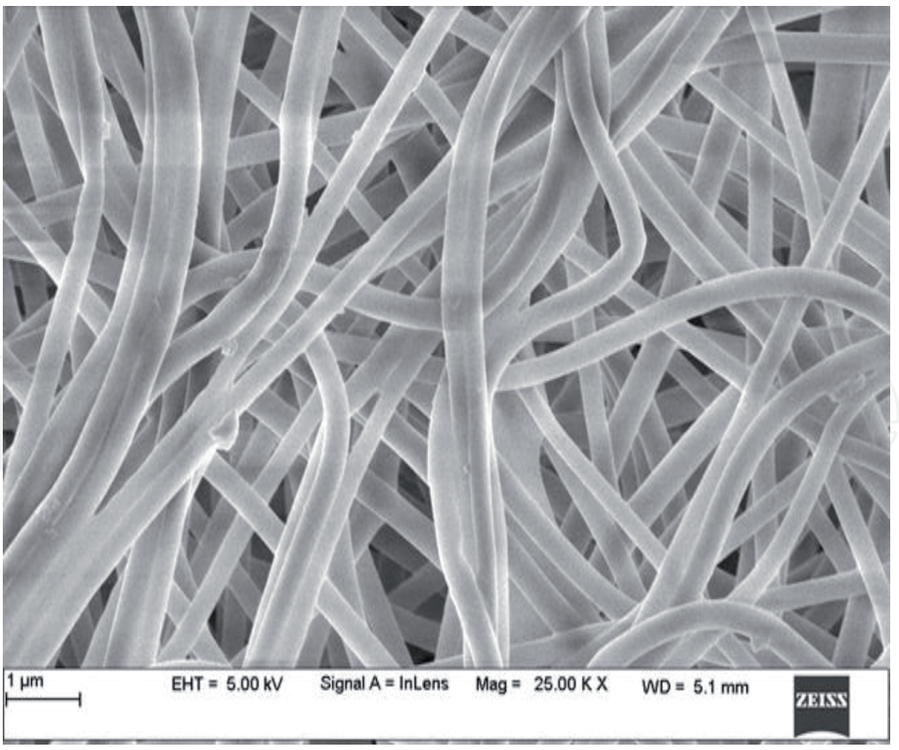


Figure 3.
SEM image of the PVA film, scale 1 µm and 40K X magnification.

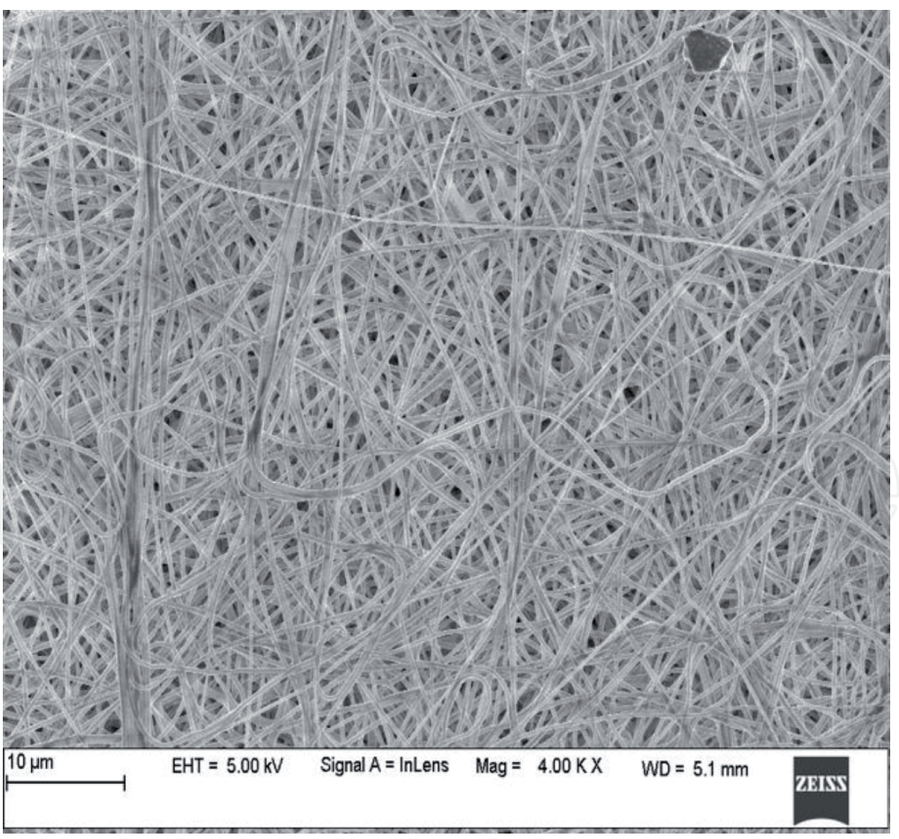


Figure 4.
SEM image of the PVA-HA film, scale 10 µm and 4K X magnification.

electron microscopy (SEM) was used to investigate the morphology of different types of films. To analyze complex structural variations upon undergoing electrospinning, and the interactions between HA and PVA. Due to abundant -OH group

Sample	Area (mm^2)	Tensile strength (MPA)	Young Modulus (GPA)	Strain at break (MPA)	Stress at break (MPA)	Percentage total elongation at fracture
Trial 1	0.42	6.06311	29.12233	0.355376	2.018833	35.5378
Trial 2	0.14	24.954	217.310	0.960583	12.66005	96.0584

Table 1.
UTM analysis for PVA films.

present in PVA and HA nanorods, they strongly interact via H-bonding within the electrospun HA-PVA nanocomposite fibres, improving thermal property.

In PVA film, at a scale of 10 μm, EHT = 5.00 kV and 4 K X magnification, the PVA film appeared like thin fibres arranged very closely (**Figure 2**). By Increasing the magnification to 40 K X and decreasing the scale to 1 μm, we observed combination of fibres with different sizes (**Figure 3**) ranging from 130 to 200 nm. In PVA-HA Film, at a scale of 10 μm, EHT = 5.00 kV and 4.00 K X magnification, the fibres are densely populated having an average size of 300 nm. Structure of nanofibres exist with relatively uniform dispersion with PVA.

After the successful binding of PVA with HA, carbon nanotubes were added with PVA to increase the strength of the film and give stability to the unwoven fibres. Since it has remarkable mechanical, thermal properties and outstanding electrical conductivity. It is much stronger and more functional fibres than current traditional industrial fibres. Hence in PVA-CNT film SEM gives successful result producing nano fibres, at a scale of 10 μm, EHT = 5.00 kV and 4.00 K X magnification (**Figure 4**) by increasing the magnification the fibres can be observed clearly.

6.2 Universal testing machine (UTM) analysis

Test is carried out to obtain the stress and strain at break and determine tensile strength of the fibres. This test is useful in getting the information of mechanical behavior and the engineering performance of the material. The first trial carried out on the film showed reduced tensile strength of 6.0631 MPA with young's modulus of 29.122 GPA. But the trial gave an improved result with increased tensile strength of 24.954 MPA and 217.310 GPA.

The percentage of elongation at fracture was also seen increasing from trial 1 to trial 2 from 35.5378 to 96.0584 respectively **Table 1**.

7. Conclusions

We prepared HA/PVA/CNT nanofibers nanocomposite via in situ co-precipitation method through electrospinning process. The produced nanofibers are a biomimetic composite of 200-300 nm. UTM analysis of the produced fibers proved to have better young modulus and tensile strength. The nano-bio materials are supposed to stimulate cell adhesion to the ECM matrix, and subsequent mineralization and tissue regeneration. The electrospun HA/PVA/CNT nanofibers shall have the potential for bone tissue engineering.

A nano-biomaterials-based scaffold shall have a high aspect ratio, better wettability, improved mechanical properties. These properties of nano-biomaterial-based scaffold make them desirable for tissue engineering. The scaffold-based on HA/PVA/CNT nanofibers are expected to have better regeneration capacity in vivo, compared to the conventional counterpart. Bone tissue engineering research requires the combination of mechanically strong biocompatible materials, which is essential in

cell adhesion and proliferation. Hence, we propose the combination of these materials with stem cells and various growth factors as the future strategies in the field of biomedical and bone tissue engineering. We propose the two tools (i) nano-scaffoldings, and (ii) cell micro-encapsulation for equal distribution of the cells within the scaffolding. Furthermore, this research shall be validated by in vitro & in vivo studies to establish the efficacy of the tailor-made nano-bio materials for orthopedic and craniofacial applications.

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References

[1] Weiner S, Traub W. Bone structure: from ångstroms to microns. The FASEB journal. 1992 Feb;6(3):879-85.

[2] Rho JY, Kuhn-Spearing L, Zioupos P. Mechanical properties and the hierarchical structure of bone. Medical engineering & physics. 1998 Mar 1;20(2):92-102.

[3] Fratzl P. Characterizing natural fibre composites with hierarchical structure. Fibre Diffraction Rev. 2002 Apr;210:31-9.

[4] Park J B and Lakes R S 1992 Biomaterials 2nd edition (New York: Plenum) p 194

[5] Ravaglioli A, Krajewski 'Bioceramics A. materials properties applications'.

[6] Elliott JC. Handbook of Structure and Chemistry of the Apatite and Other Calcium Orthophosphates. Vol. 18.

[7] Butler WT, Ritchie HE. The nature and functional significance of dentin extracellular matrix proteins. International Journal of Developmental Biology. 2003 Feb 1;39(1):169-79.

[8] Park J, Lutz R, Felszeghy E, Wiltfang J, Nkenke E, Neukam FW, Schlegel KA. The effect on bone regeneration of a liposomal vector to deliver BMP-2 gene to bone grafts in peri-implant bone defects. Biomaterials. 2007 Jun 1;28(17):2772-82.

[9] Kocialkowski A, Wallace WA, Prince HG. Clinical experience with a new artificial bone graft: preliminary results of a prospective study. Injury. 1990 May 1;21(3):142-4.

[10] Dujardin E, Mann S. Bio-inspired materials chemistry. Advanced Materials. 2002 Jun 5;14(11): 775-88.

[11] Kikuchi M, Itoh S, Ichinose S, Shinomiya K, Tanaka J. Self-organization mechanism in a bone-like hydroxyapatite/collagen nanocomposite synthesized in vitro and its biological reaction in vivo. Biomaterials. 2001 Jul 1;22(13):1705-11.

[12] Yamaguchi I. Preparation and mechanical properties of chitosan/ hydroxyapatite nanocomposites. Key Engineering Materials. 2001 Jul 1;192.

[13] Chen F, Wang ZC, Lin CJ. Preparation and characterization of nano-sized hydroxyapatite particles and hydroxyapatite/chitosan nano-composite for use in biomedical materials. Materials letters. 2002 Dec 1;57(4):858-61.

[14] Liao SS, Cui FZ, Zhang W, Feng QL. Hierarchically biomimetic bone scaffold materials: nano-HA/collagen/PLA composite. Journal of Biomedical Materials Research Part B: Applied Biomaterials: An Official Journal of The Society for Biomaterials, The Japanese Society for Biomaterials, and The Australian Society for Biomaterials and the Korean Society for Biomaterials. 2004 May 15;69(2):158-65.

[15] Deng X, Hao J, Wang C. Preparation and mechanical properties of nanocomposites of poly (D, L-lactide) with Ca-deficient hydroxyapatite nanocrystals. Biomaterials. 2001 Nov 1;22(21):2867-73.

[16] Jie, W., Yubao, L. and Weiqun, C., 2003. A study on nano-composite of hydroxyapatite and polyamide. Journal of materials science, 38(15), pp.3303-3306.

[17] Huang M, Feng J, Wang J, Zhang X, Li Y, Yan Y. Synthesis and characterization of nano-HA/PA66 composites. Journal of Materials

Science: Materials in Medicine. 2003 Jul;14(7):655-60.

[18] Hartgerink JD, Beniash E, Stupp SI. Self-assembly and mineralization of peptide-amphiphile nanofibers. Science. 2001 Nov 23;294(5547):1684-8.

[19] Marouf HA, Quayle AA, Sloan P. In vitro and in vivo studies with collagen/hydroxyapatite implants. International Journal of Oral & Maxillofacial Implants. 1990 Jun 1;5(2).

[20] Du C, Cui FZ, Zhu XD, de Groot KA. Three-dimensional nano-HAp/collagen matrix loading with osteogenic cells in organ culture. Journal of Biomedical Materials Research: An Official Journal of The Society for Biomaterials, The Japanese Society for Biomaterials, and The Australian Society for Biomaterials. 1999 Mar 15;44(4):407-15.

[21] Reneker DH, Chun I. Nanometre diameter fibres of polymer, produced by electrospinning. Nanotechnology. 1996 Sep 1;7(3):216.

[22] Kim GM, Wutzler A, Radusch HJ, Michler GH, Simon P, Sperling RA, Parak WJ. One-dimensional arrangement of gold nanoparticles by electrospinning. Chemistry of materials. 2005 Oct 4;17(20):4949-57.

[23] Dror Y, Salalha W, Khalfin RL, Cohen Y, Yarin AL, Zussman E. Carbon nanotubes embedded in oriented polymer nanofibers by electrospinning. Langmuir. 2003 Aug 19;19(17):7012-20.

[24] Kim GM, Michler GH, Pötschke P. Deformation processes of ultrahigh porous multiwalled carbon nanotubes/polycarbonate composite fibers prepared by electrospinning. Polymer. 2005 Aug 23;46(18):7346-51.

[25] Khalid P., Suman V. B., "Carbon nanotubes integrated nanocomposites membranes for purification of water", Elsevier, Amsterdam, Netherlands, Aquananotechnology: Applications of Nanomaterials for Water Purification, 1st Edition, 2020, ISBN: 978-0-12-821141-0.

[26] Khalid P., Suman V. B., "Risk Management and Regulatory Aspects of Carbon Nanomaterials", Elsevier, Amsterdam, Netherlands, Carbon Nanomaterials for Agri-food and Environmental Applications, 1st Edition, 2019, ISBN 978-01-28197-86-8.

[27] Khalid P., Suman V. B., "Quality Control and Risk Management of Carbon Nanomaterial", INTECHOPEN LIMITED, London, UK, Perspective of Carbon Nanotubes, 2019, ISBN 978-1-78984-402-3.

[28] Khalid P., Suman V. B., "Current Toxicological Knowledge of Carbon Nanotubes", Research India Publication, Advanced Engineering Research and Applications (Book Code: AERA), 2017, ISBN No. 978-93-84443-48-1.

[29] Khalid P., Hussain M. A., Rekha P. D., Suman V. B., Arun A. B., "Modifications of Carbon Nanotube for Bio-Applications and Toxicity Evaluation", Bloomsbury Publication, India, Nano Biomaterials, 2012, ISBN 978-93-82563-37-2.

[30] Parwez K, Budihal SV. Optimisation of carbon nanotubes for advanced diagnosis and biomedical application. International Journal of Nanoparticles. 2019;11(3):217-38.

[31] Khalid P, Suman VB. Carbon nanotube-hydroxyapatite composite for bone tissue engineering and their interaction with mouse fibroblast L929 In Vitro. Journal of Bionanoscience. 2017 Jun 1;11(3):233-40.

[32] Koonprasert S, Channgam N. Global stability and sensitivity analysis of SEIQR worm virus propagation model with quarantined state in mobile

internet. Global Journal of Pure and Applied Mathematics. 2017;13(7):3833-50.

[33] Khalid, P., Hussain, M.A., Rekha, P.D. and Arun, A.B., 2015. Carbon nanotube-reinforced hydroxyapatite composite and their interaction with human osteoblast in vitro. Human & experimental toxicology, 34(5), pp.548-556.

[34] Parwez K, Budihal SV. Carbon nanotubes reinforced hydroxyapatite composite for biomedical application. Journal of Bionanoscience. 2014 Feb 1;8(1):61-5.

[35] Khalid P, Hussain MA, Rekha PD, Sanal C, Suraj S, Rajashekhar M, Suman VB, Arun AB. Interaction of Carbon Nanotubes Reinforced Hydroxyapatite Composite with Bacillus subtilis, *P. aeruginosa* and *C. albicans*. Indian Journal of Science and Technology. 2014 May 1;7(5):678.

[36] Khalid P, Hussain MA, Rekha PD, Suman V, Arun A. Modification of Carbon Nanotubes for bioapplications and toxicity evaluations. J. Environ. Nanotechnol. 2013 Jan 1;2(1):42-6.

[37] Khalid P, Hussain MA, Rekha PD, Arun AB. Synthesis and characterization of carbon nanotubes reinforced hydroxyapatite composite. Indian Journal of Science and Technology, 2013, 6(12), pp.5546-51. DOI: 10.1007/s12666-017-1150-6