Global Journals LaTeX JournalKaleidoscope™
Artificial Intelligence formulated this projection for compatibility purposes from the original article published at Global Journals. However, this technology is currently in beta. *Therefore, kindly ignore odd layouts, missed formulae, text, tables, or figures.*

Fabrication and Characterization of Porous Nanohydroxyapatite/Chitosan-Cellulose Composite Scaffold for Biomedical Application

G.M. Pavithra

Received: 14 June 2021 Accepted: 30 June 2021 Published: 15 July 2021

Abstract

Bones are stiff structures that upkeep and guard several body parts of the physique. A medical technique entitled bone grafting substitutes misplaced bone to overhaul bone fractures that are very intricate, Otherwise, that does not cure precisely.Methods: Several scaffold formulations are prepared (S1, S2, S3, and S4) using various polymers. The prepared scaffold was studied for their weight loss, swelling ability, X-Ray Diffraction (XRD), Scanning Electron Microscopy (SEM) Electron Dispersive X-Ray Analysis, Transmission electron microscopy (TEM), Fourier Transform Infrared Spectroscopy (FT-IR), optical microscopy, in vitro release studies, and in vitro antimicrobial studies.

Index terms— bone grafting, scaffolds, hydroxyapatite, ofloxacin, chitosan.

1 Introduction

n the past two decades, tissue engineering by bone regeneration has become an alternative method used to overcome the shortcomings of conventional bone defect treatments [1]. Bones are upkeep and guard various organs of the body. Damage induces a significant decrease in the quality of our life. A medical technique called bone grafting substitutes lost bones to patchup bone fractures, which are very difficult, imparting substantial health hazards to a patient, or flop to cure appropriately. The grafts may be autologous, allograft, or synthetic. Many of the grafts get reabsorbed and substituted when the normal bone reconciles over some time. The doctrines in fruitful grafts include osteoconduction, osteoinduction, and osteogenesis [2].

The progress inthe medical discipline has upgraded biomaterials role in substituting injured tissue, organs and enhancing their functions. Bone tissue engineering is a novel treatable practice for bone grafting [3]. The tissue engineering research is implemented mainly in two fields: osteo and dental applications. This technique implants scaffolds, which give mechanical strength in the crackzones. The scaffold remains as a momentary medium for cell multiplication until fresh tissue is entirely revived [4].

Hydroxyapatite (HAP) is one of the apatite materials that have a significant inorganic constituent of teeth and bone, which has high biocompatible and bioactive properties and hence employed in bone tissue engineering. Its flow property strength is very little than those required for bone tissue engineering materials and has a tend to migrate from implant sites. These limitations can be overwhelmed by combining hydroxyapatite with organic constituents, thus mimicking the ECM of bone [5].The contagions allied with the implantation recurrently minimize the usage of biomaterials in humans. Bacteria trigger the patient's immune system forming a protective film by sticking onto biomaterial exterior. To avoid these complications, ofloxacin which possesses antibacterial activity, has been incorporated in this biomaterial [6]. Thus, the present research work was intended towards the formulation of nano biocomposite scaffold of hydroxyapatite-chitosan-cellulose. Five formulations namely, S1, S2, S3, S4, and S5, was developed. These five formulations are initially characterized for various properties. The optimized formulation, i.e.,. S5, was characterized by analytical techniques.

2 II.

3 Materials and Methods

4 a) Materials

Hydroxyapatite, Chitosan, Sodium Carboxy Methyl Cellulose, Carboxy Methyl Cellulose, Hydroxy Propyl Methyl Cellulose, Ofloxacin and acetic acid were purchased from Sastha Scientific Services, Chennai.

5 III.

6 Methodology a) Preparation of Hydroxy Apatite Nanoparticles

The orthophosphoric acid solution was added drop by drop into calcium hydroxide solution under magnetic stirring at 70°C for 3 hours. The mixture is stirred until a clear and homogenous solution formed, and then sodium hydroxide solution was added to this solution until pH value was maintained at 10. The white precipitates were left for 4 hours. The obtained nanoparticles were parted, clarified with deionized water, and dried under ambient atmosphere. It was then

7 b) Fabrication of Scaffold

Hydroxy Propyl Methyl Cellulose(HPMC) and 100 mg of ofloxacin drug were dissolved in water using a mechanical stirrer until a homogenous solution was formed. Secondly, chitosan was solubilized in 2% acetic acid, which was instilled dropwise into the HPMC mixture. It is then mixed at 500 rpm. These mixtures were added to the above-formed nanoparticles. The stirring is kept for 24hrs and, the gel formed was then transferred into the tissue culture dish and cooled at -24°C for 24 hrs and lyophilized to form scaffolds. These

8 c) Preparation of Five Formulations of Scaffold

By experimenting with different polymers, five formulations of the scaffold was prepared namely, S1, S2, S3, S4, and S5.

9 IV.

10 Characterization Studies a) Calibration Curve of Ofloxacin

The calibration curve of ofloxacin was performed using various concentrations of ofloxacin, as given in Figure no.1 [11].

11 b) Fabrication of the Nanocomposite Scaffold

The scaffold was prepared as per the procedure described in Figure no.2. The quantities of the ingredients in each scaffold are described in

12 d) Swelling Ability

The parched mass of the scaffolds was represented as W i . Parched scaffolds were submerged in Phosphate Buffer solution at 37°C for 24 hours. Later, the scaffolds were removed from PBS solution, and its damp mass was denoted as W f . Swelling ability data was depicted in Table no.3. Swelling Ability (%) = [(W f -W i) /W i] x100 e) Porosity Measurement

W d was used to represent the dry weight of the scaffolds, while W l designated the mass of the scaffolds after immersing in ethyl alcohol for five minutes. After slight parching over the shallow area, W w was recorded. The porosity data is described in Table no.4 [12].Porosity (%) = (W w -W d) / (W w -W l) x 100 f) FT-IR Analysis

The spectra of the Chitosan, HPMC, Ofloxacin, and the optimized F5 formulation were documented by means of potassium bromide pellet method in the FT-IR spectrophotometer (JASCO 4100 type A) within the range of 4000cm-1 to 400cm-1 [13].

V.

13 Surface Analysis a) Scanning Electron Microscopy (SEM)

The powdered sample was taken and mounted on a double side carbon tape, which was fixed to sample specimen stub. The SEM (QUANTA FEG) instrument is used for analysis. The SEM images were described in Figure no.4 [14].

14 b) Optical Microscopy

MOTIC digital microscope is used to image the scaffold at 10X and 40X, as given in Figure no.5.

15 c) Transmission Electron Microscopy (TEM)

TEM studies were useful in examining the morphological and crystalline arrangements of the scaffold. The principle employed to view the scaffolds is high-resolution transmission electron microscopy (HRTEM). The scaffold's (20 µl) solution was taken. On the carbon-coated side of the copper lattice, the mixture was dripped. At room temperature for few hours, the lattice was dehydrated. The grid was then placed in the sample holder and mounted in the instrument. The instrument TECHNAI T20 was used for the analysis. The TEM images were given in Figure no.6 [15].

16 d) Electron Dispersive X-Ray Analysis

The elements present in the scaffold were estimated using EDAX analysis. It is given in Figure no.7 [16]. B heated in an electric furnace at 700°C to obtain pure nanoparticles [7,8].© 2021 Global Journals

scaffolds were cross-linked with CaCl 2 solution for 30 minutes, followed by sopping in ethanol for 10 minutes. Finally, the scaffold was clarified with water and another timely ophilized [9,10].

17 VI.

In-vitro Release Studies 100µg of the scaffold was pondered from each of the five formulations primed in different test tubes. To this, pH 7.4 phosphate buffer medium was added and placed in an orbital shaker. The quantity of ofloxacin expelled out from the scaffolds was assessed by amassing buffer medium from the test tubes and supplanting with fresh buffer at 30 minutes' intervals for 5 hours. The amount expelled out was recorded at 294 nm. The discharged amount was ascertained from the standard curve. From this percentage, drug release was calculated, and percentage drug release as plotted versus time. The in-vitro drug release graph was depicted in Figure no.9 [17].

VII.

18 In-vitro Antibacterial activity a) Agar Disc Diffusion Method i. Preparation of Inoculum

On agar slant, cultures were conserved at 4°C. By relocating a coil of cells from the cultures to test tubes, lively cultures were developed. The anti-septic action was ascertained by the agar disc diffusion technique.

19 ii. Antibacterial Activity

The antiseptic activity was ascertained by the well diffusion method on Muller Hinton agar (MHA) medium. MHA was solubilized in purified water, and the medium was sterilized after the addition of agar. Then, the media was transferred into disinfected Petri plates and solidified. By using disinfected swab saturated with the bacterial suspension, the inoculums were spread on the plates. To the wells made, 100,200, 400µg of (F5), 50 µl negative control (HCl), and positive control of streptomycin suspension were added on respective wells. These plates were gestated at 37ºC for a day. The area of inhibition was then recorded. The results were depicted in Table no

20 Results and Discussion

21 a) Calibration Curve of Ofloxacin

22 c) Weight Loss

From the above shown Fig. 3 and Table 3, Scaffold S1 has a maximum weight loss of 8 % during the study. The scaffold S3 showed less weight loss compared to S2. Scaffold S4 showed the minimum loss of weight (2.7 %) in four weeks and had the less degradation [19]. The swelling was similar in all the scaffold formulations due to constant hydroxyapatite and chitosan concentrations, as given in table no. **??** [20]. Table 3 shows the Parched mass (W i in g), damp mass (W f in g) and swelling ability (%) of scaffold formulations.

23 e) Porosity Measurement

The porosity of the scaffold formulations was similar to one another, as given in table no.4. 4 reveals the parched mass (W w in g), dry weight (W d in g), dipped mass (W l in g) and porosity (%) of the scaffold formulations.

24 f) FT-IR Analysis

The results of the analysis showed various stretching, bending, and rocking vibrations based on the groups present. All the spectra indicated that there are no significant drug-excipient interactions.

25 IX.

26 Surface Analysis a) Scanning Electron Microscopy (SEM)

The images exhibit that the scaffold has an elongated surface which is shown in figure no.4.

27 d) Energy Dispersive X-Ray Analysis

The scaffold contains oxygen(O), carbon(C), calcium(Ca), phosphorus(P), magnesium (Mg) and chlorine(Cl) at 50.80%, 24.93%, 24.64%, 8.19%, 0.50% and 0.36% respectively as shown in Figure no.7 [22]. Fig. **??**: EDAX analysis Fig. **??** shows the presence of various elements and their composition of S4 scaffold.

28 e) X-Ray Diffraction (XRD) Analysis

The peaks were obtained at 2? level at positions 27. **??**1, 29.15, 30.65, 34.35, 37.

29 In-vitro Release Studies

From the Figure no.9, the scaffold S4 showed an initial burst release succeeded by a persistent release and the release rate was found to be 100% at the end of 8hours, whereas scaffolds S2 and S3 showed release of 62%, and 82% at the end of 8 hours study. However, S5 showed a sustained release profile over an extended period of study of upto 24 hours. Hence, the formulation S5 has been optimized for characterization [23]. From the report of the antibacterial activity of the formulated scaffold as shown in Table no.5 and Figure no.10, it was found that the scaffold with various concentrations 100µg, 200µg and 400µg when compared with standard positive and negative control, showed maximum zone of inhibition of 26mm, 32mm and 34mm respectively. Hence the prepared scaffold exhibits antibacterial activity [24].

30 Conclusion

The scaffold is a versatile bioactive product among wound dressing materials, whose production is flexible and economical. The present work was aimed towards fabricating a scaffold containing hydroxyapatite using various polymers like chitosan, carboxy methylcellulose (CMC), sodium carboxy methylcellulose (SCMC), and hydroxypropyl methylcellulose (HPMC) by freeze-drying technique by incorporating Ofloxacin as an antimicrobial agent. Five formulations, namely S1, S2, S3, S4, and S5, were prepared using various combinations of the polymers mentioned. The prepared scaffolds were studied for their characteristic properties like weight loss, swelling ability, porosity, and in-vitro drug release studies. The optimized formulation (S5) was characterized by SEM, optical microscopy, TEM, EDAX, XRD, FT-IR, and in-vitro antibacterial activity.

Due to the greater water acceptance, sufficient porosity, improved antibacterial activity, and extended drug release, the hydroxyapatite-chitosan-HPMCofloxacin scaffold would be a hopeful biomaterial for bone tissue engineering. From this research, it was concluded that the nano-composite scaffold is a viable alternative to existing conventional dosage forms, which lead to improved bioactivity and a promising biomaterial for bone tissue engineering in case of administration affords resulting in better patient compliance and costeffective therapy in the field of biomedical application.

31 Conflicts of Interest

[1]

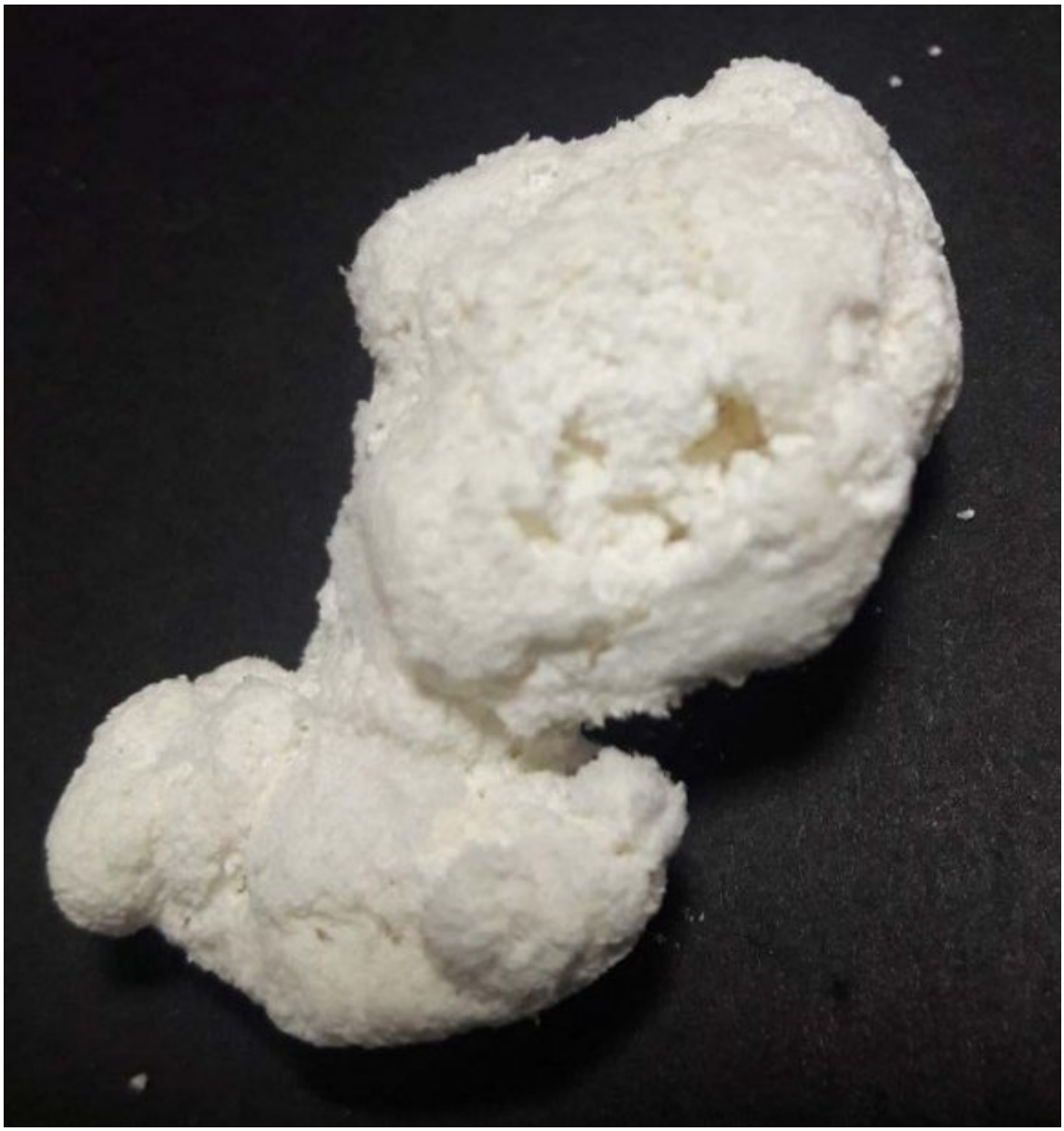


Figure 1: Fabrication

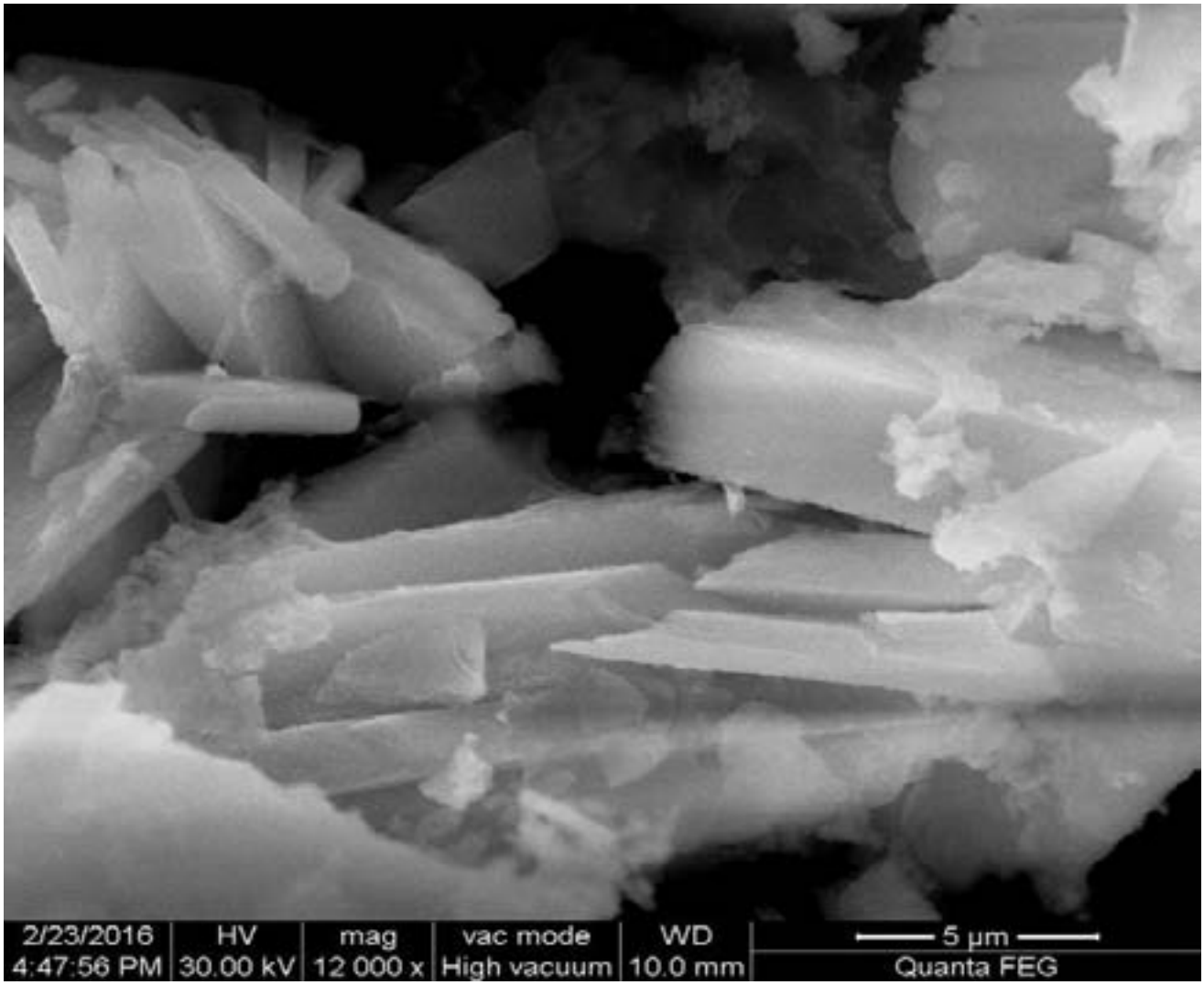

2/23/2016
4:47:56 PM
HV
30.00 kV
mag
12 000 x
vac mode
High vacuum
WD
10.0 mm
5 µm
Quanta FEG

Figure 2:

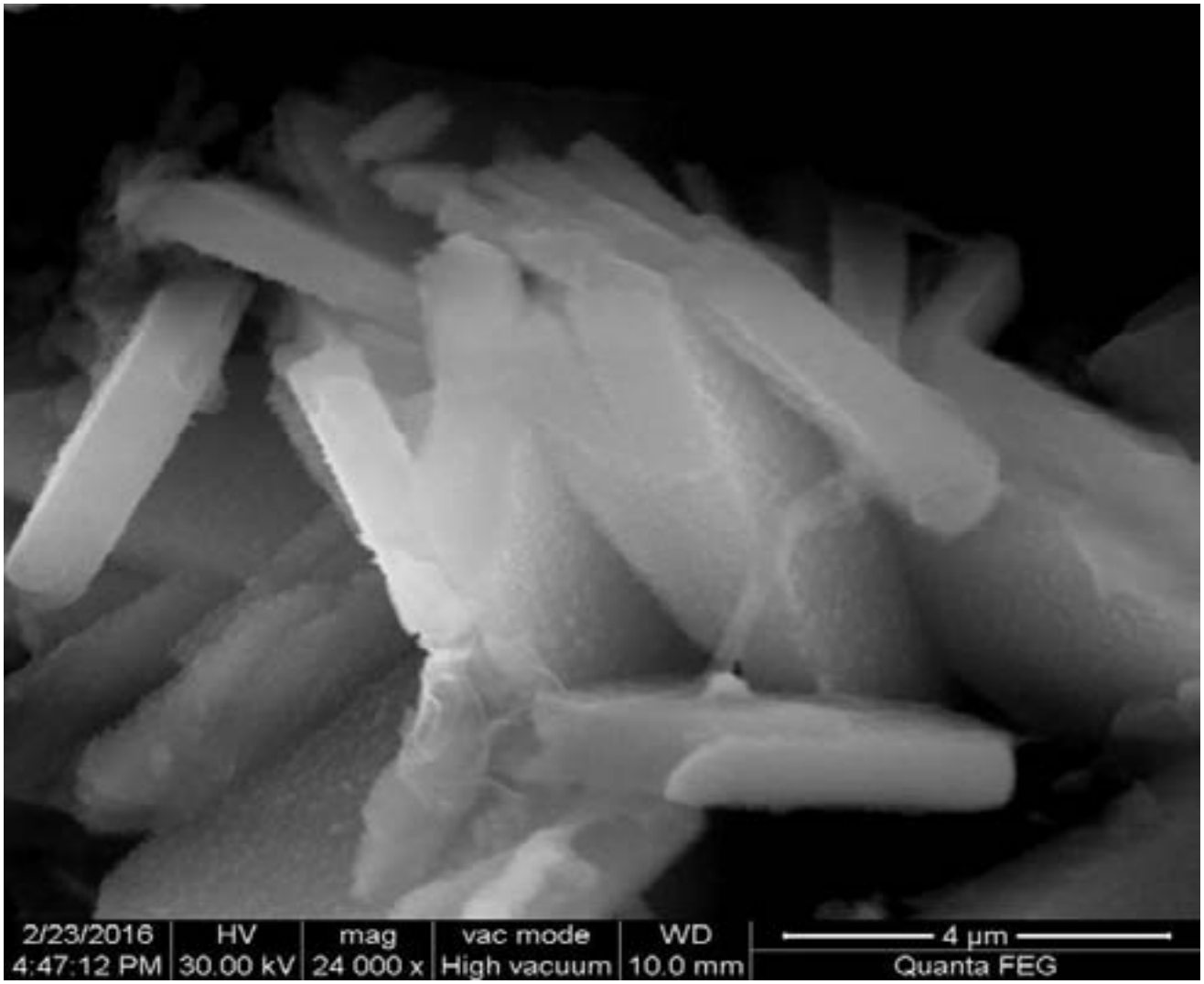
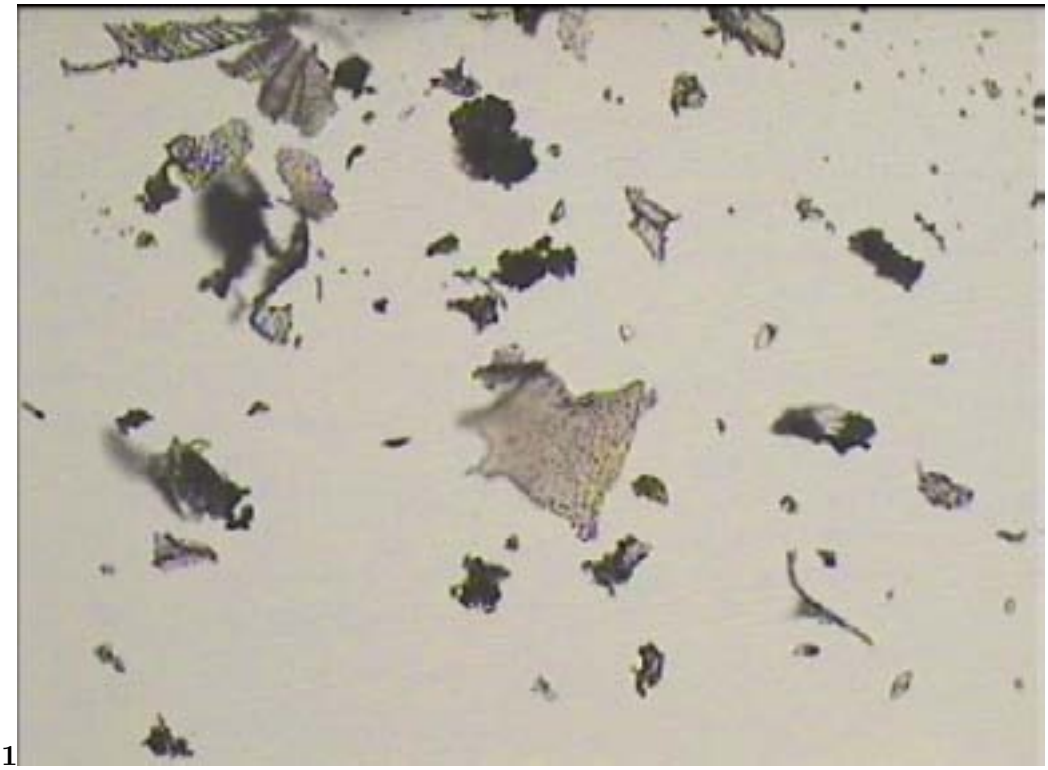

2/23/2016
4:47:12 PM
HV
30.00 kV
mag
24 000 x
vac mode
High vacuum
WD
10.0 mm
4 µm
Quanta FEG

Figure 3:



1

Figure 4: Fig. 1 :

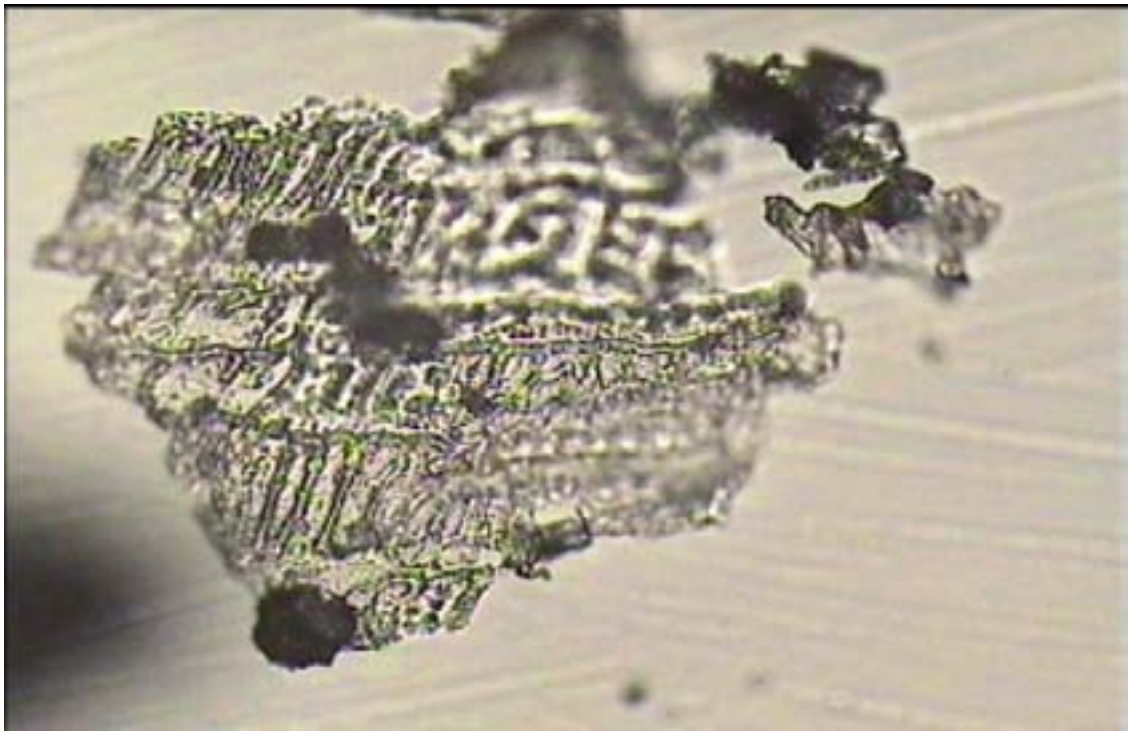
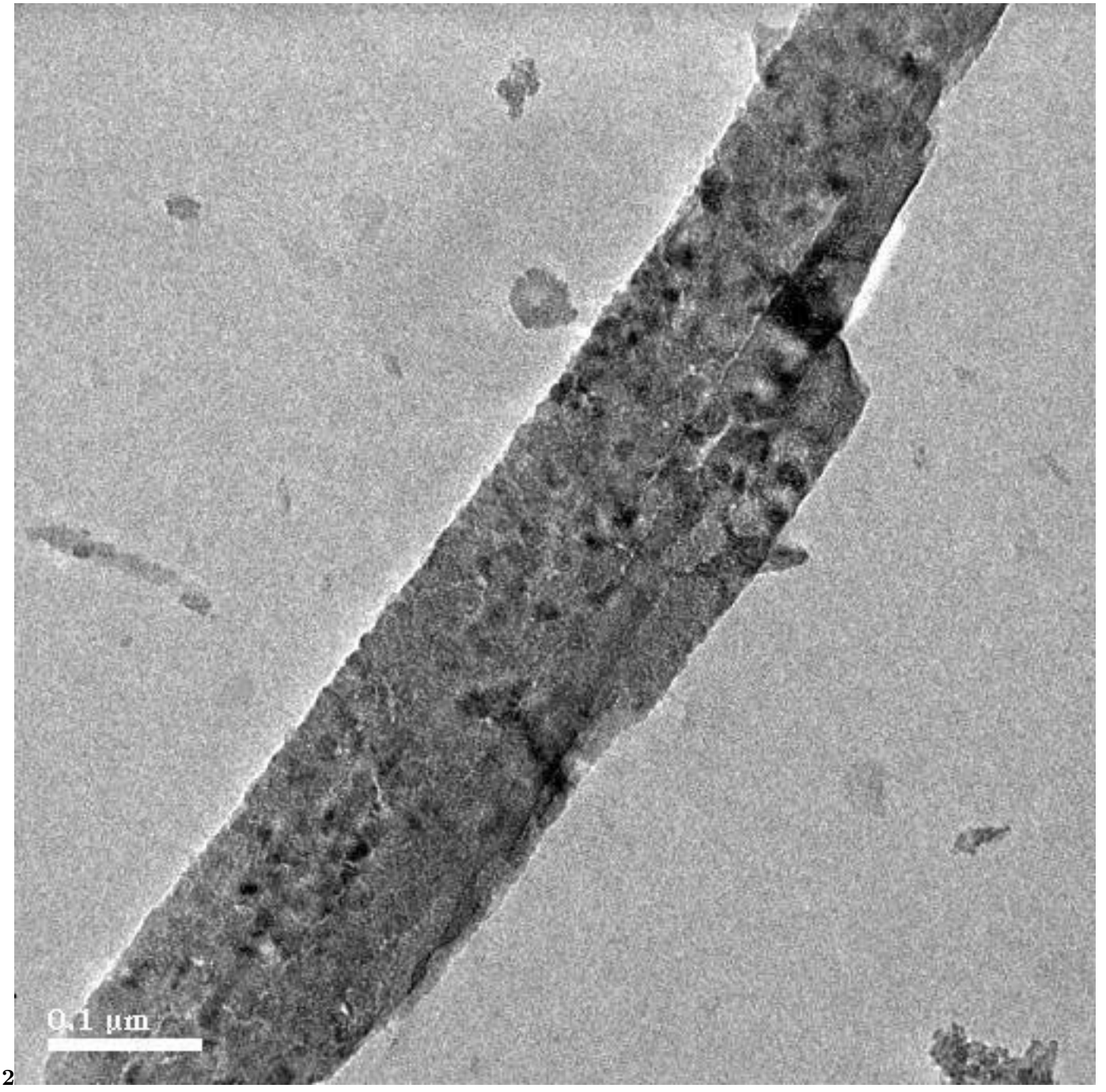
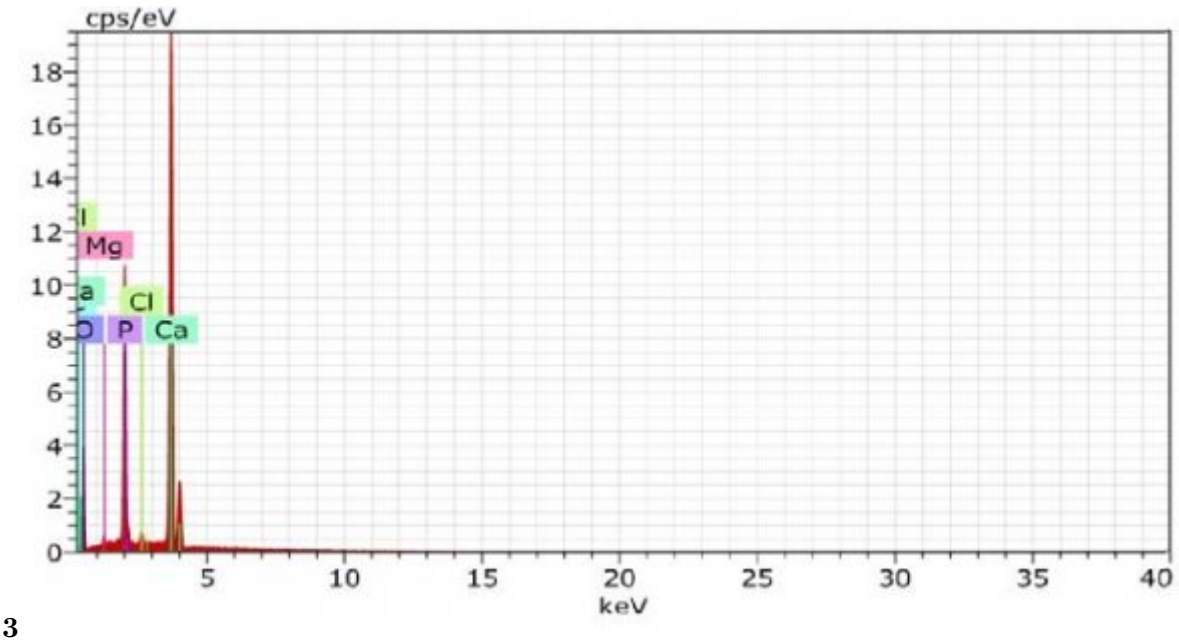


Figure 5: Fabrication



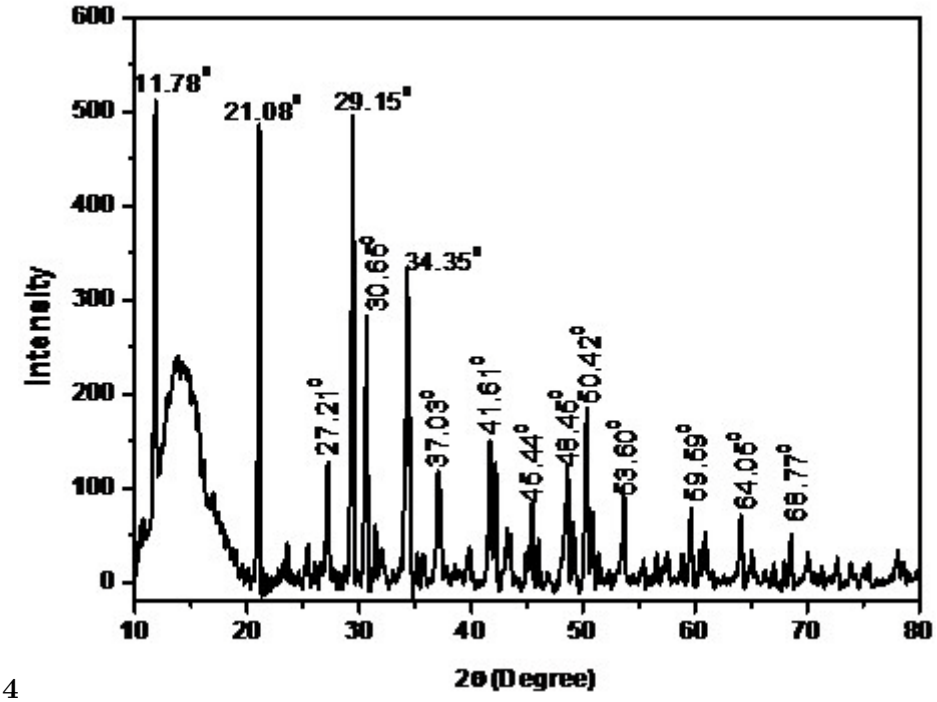
2

Figure 6: Fig. 2 :



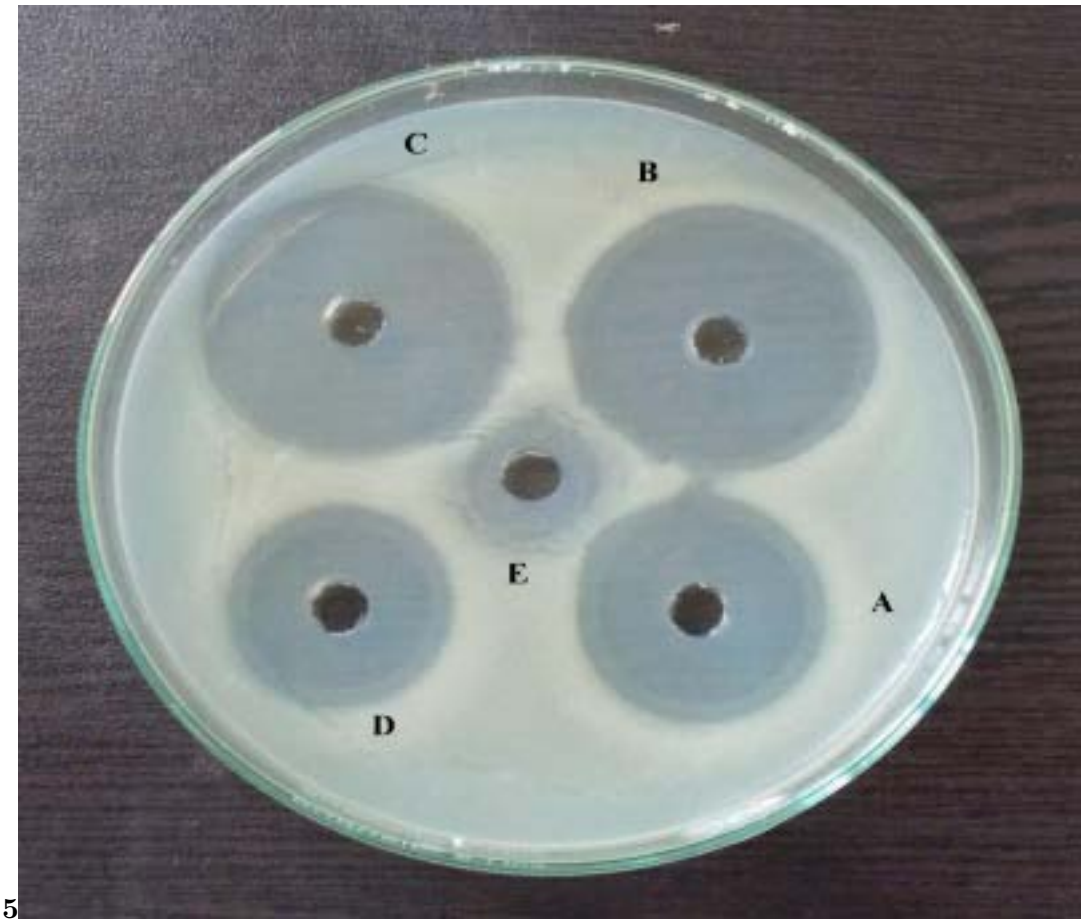
3

Figure 7: Fig. 3 :



4

Figure 8: Fig. 4 :



5

Figure 9: Fig. 5 :

no

.1.
c) Weight Loss
By imbibing the scaffolds in Simulated Body Fluid (SBF), the weight losses of the five scaffold formulations are conceded.
Weight Loss (%) = [(W o -W t) / W o] x 100

Figure 10: Table no

1

Formulation Code	S1 (mg)	S2 (mg)	S3 (mg)	S4 (mg)
Hydroxyapatite	100	100	100	100
Chitosan	30	30	30	30
SCMC	-	10	-	-
CMC	-	-	10	-
HPMC	-	-	-	10
Ofloxacin	100	100	100	100
2% Acetic Acid	50ml	50ml	50ml	50ml
Water	100ml	100ml	100ml	100ml

[Note: Note: Details the composition of S1, S2, S3, and S4 formulations.© 2021 Global Journals]

Figure 11: Table 1 :

2

Time (d)	S1 (%)	S2 (%)	S3 (%)	S4 (%)
1	1.5	0.2	0.1	0.0
3	2.7	1.2	0.9	0.0
7	3.9	2.2	1.7	0.5
15	5.4	3.1	2.4	1.2
21	6.9	4.2	3.3	1.9
28	8.0	5.1	4.0	2.7

[Note: Note: Represents the loss of weight in % of scaffolds at predetermined time intervals for 28 days.]

Figure 12: Table 2 :

3

Formulation Code	W i (g)	W f (g)	Swelling Ability (%)
S1	1.00	2.40	140
S2	1.00	2.60	160
S3	1.00	2.50	150
S4	1.00	2.90	190

Figure 13: Table 3 :

4

Formulation Code	W w (g)	W d (g)	W l (g)	Porosity (%)
S1	0.58	0.25	1.15	57.89
S2	0.59	0.25	1.19	56.66
S3	0.62	0.25	1.20	63.79
S4	0.65	0.25	1.24	67.79

Table

Figure 14: Table 4 :

5

S.No.	Microorganisms	Zone of Inhibition (mm) 100µg	200µg	400µg	HCl (negative control)	Streptomycin 15 µg (positive control)
1	Escherichia coli	26	32	34	23	16

Figure 15: Table 5 :

[Zhi-Cai Xing et al. ()] , Wan Zhi-Cai Xing , Jiang Meng , Sungmo Yuan , Moon , Inn-Kyu Yongsoojeong , Kang . *In Vitro Assessment of Antibacterial Activity and Cytocompatibility of Quercetin-Containing PLGA Nanofibrous Scaffolds for Tissue Engineering.J. Nanomater* 2012. p. .

[Lane et al. ()] 'Biosynthetic bone grafting'. J M Lane , E Tomin , Mpg Bostrom . *Clin. Orthop. Relat. Res* 1999. 367 p. .

[Wattanutchariya and Changkowchai ()] *Characterisation of Porous Scaffold from Chitosan-Gelatin/Hydroxyapatite for Bone Grafting*, Wassanai Wattanutchariya , Whattanapong Changkowchai . IMEC. 2: 2210. 2014.

[Robisnson and Watson ()] 'Collagen-crystal relationship in bone as seen in the electron microscope'. R A Robisnson , M L Watson . *Anatom. Rec* 1952. 114 p. .

[Yao et al. ()] 'Dual-Drug-Loaded Silk Fibroin/PLGA Scaffolds for Potential Bone Regeneration Applications'. Jihang Yao , Yilong Wang , Wendi Ma , Wenying Dong , Mei Zhang , Dahui Sun . *J. Nanomater* 2019. p. .

[Rajzer et al.] *Electrospun polymer scaffolds modified with drugs for tissue engineering C*, Izabella Rajzer , Oscar Elzbietamenaszek , Castano . 77 p. .

[Scimeca et al. ()] 'Energy Dispersive X-ray (EDX) microanalysis: A powerful tool in biomedical research and diagnosis'. Manuel Scimeca , Simone Bischetti , Rita Harpreet Kaur Lamsira , Elena Bonfiglio , Bonanno . *Eur J Histochem* 2018. 62 p. 2841.

[Seyedaliseyedmajidi et al. ()] 'Evaluation of antibacterial properties of hydroxyapatite/bioactive glass and fluorapatite/bioactive glass nanocomposite foams as a cellular scaffold of bone tissue'. Ramazan Seyedaliseyedmajidi , Maryam Rajabnia , Seyedmajidi . *J. Lab* 2018. 10 p. .

[Yaszemski et al. ()] *Evolution of bone transplantation: Molecular, cellular and tissue strategies to engineer human bone*, M J Yaszemski , R G Payne , W C Hayes , R Langer , A G Mikos . 1996. 17 p. .

[Gervaso et al. ()] 'High-Performance Hydroxyapatite Scaffolds for Bone Tissue Engineering Applications'. Francesca Gervaso , Francesca Scalera , Sanoshkunjalukkal Padmanabhan , Alessandro Sannino , Antonio Licciulli . *Int. J. Appl. Ceram. Technol* 2012. 9 p. .

[Algul et al. ()] 'In vitro release andIn vivo biocompatibility studies of biomimetic multilayered alginate-chitosan/?-TCP scaffold for osteochondral tissue'. D Algul , A Gokce , A Onal , E Servet , Dogan Ekici Al , F G Yener . *J Biomater Sci Polym Ed* 2016. 27 p. .

[Oliveira et al. ()] 'Macroporous hydroxyapatite scaffolds for bone tissue engineering applications: physicochemical characterization and assessment of rat bone marrow stromal cell viability'. J M Oliveira , S S Silva , P B Malafaya , M T Rodrigues , N Kotobuki , M Hirose . *J. Biomed. Mater. Res* 2009. 91 p. .

[Bucholz ()] 'Nonallograft osteoconductive bone graft substitutes'. R W Bucholz . *Clin. Orthop. Relat. Res* 2002. 395 p. .

[Shimojo et al. ()] 'Performance of PRP associated with porous chitosan as a composite scaffold for regenerative medicine'. A A Shimojo , A G Perez , S E Galdames , I C Brissac , M H Santana . *Sci. World* 2015. J2015. p. 396131.

[Indrani et al. ()] 'Preparation and Characterization of Porous Hydroxyapatite and Alginate Composite Scaffolds for Bone Tissue Engineering'. D J Indrani , E Budiyanto , H Hayun . *Int J App Pharms* 2018. 9 p. .

[Maji et al. ()] 'Preparation and Evaluation of Gelatin-Chitosan-Nanobioglass 3D Porous Scaffold for Bone Tissue Engineering'. Kanchan Maji , Sudip Dasgupta , Krishna Pramanik , Akalabyabissoyi . *Int. J. Biomater* 2016. 2016 p. .

[Subhashree et al. ()] 'Qualitative analysis of controlled release Ofloxacin / HPMC mucoadhesive suspension'. Sahoo Subhashree , Chakraborti Chandra Kanti , Mishra Subash Chandra , Naik Sharmistha . *Int. J. Drug Dev. Res* 2011. 3 p. .

[Farag Seddik et al. ()] 'Scaffold Development and Characterization Using CAD System'. Ahmed Farag Seddik , Ahmed Agameia , M A Sharaf , Ahmed Eldesouky . *Am. J. Biomed. Sci* 2011. 3 p. .

[Sowmya et al. ()] 'Simple U.V. spectrophotometric methods forthe estimation of ofloxacin in pharmaceutical formulations'. C Sowmya , Y Padmanabhareddy , J Ravindra Reddy , M Siva Maruthi , G Roopesh , M Santhosh Raja . *Int. J. Chem. Sci* 2010. 8 p. .

[Marksjr and Odgren ()] *Structure and development of the skeleton. Principles of bone biology*, S Marksjr , P Odgren . 2002. Chennai (TN: Elsevier Publishers. (2nd ed)

[Gerhon et al. ()] 'Structure and molecular regulation of bone matrix proteins'. R P Gerhon , N S Fedarko , T E Hefferan , P Bianco , U K Vetter , W Grzesik . *J. Bone Miner. Res* 1993. 8 p. .

[Lieberman et al. ()] 'The role of growth factors in the repair of bone. Biology and clinical applications'. J R Lieberman , A Daluiski , T A Einhorn . *J. Bone Joint Surg. Am* 2002. 84 p. 10321044.

[Westerhuis et al. ()] *Use of bone morphogenetic proteins in Traumatology*, R J Westerhuis , R L Van Bezooijen , P Kloen . 2005. 36 p. .