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Biomimetic hydroxyapatite (HAP)/ Carboxymethyl Cellulose (CMC) composite materials for bone tissue engineering applications

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Abstract

Natural bone is a complex material with a well-designed architecture. To achieve successful bone integration and regeneration, the constituents and structure of bone-repairing scaffolds need to be flexible and biocompatible. Hydroxyapatite (HAp), the main constituent of bone minerals, has excellent biocompatibility, while carboxymethyl cellulose (CMC), comprised of a three-dimensional network, has high flexibility. Therefore, CMC/HAp composites have attracted attention for the advancement of bone tissue engineering. In this work, a carboxymethyl cellulose/hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$; HAp) composite has been developed as a three-dimensional scaffold for bone tissue engineering. Scanning electron microscopy revealed that the CMC/Hap composite had a sheet-like structure. These results revealed that the amount of precipitated HAp in the CMC/HAp composites was affected by the amount of CMC used during preparation. Properties of composites can be improved at optimal filler content compared to the pure Hap in the perspective of various biomedical applications. We have characterized the surface morphology of the composite using SEM image and having with the

observed well dispersion of Hap in the Cmc phase.

Keywords: Hydroxyapatite, Carboxymethyl Cellulose, Composites, Polarization.

Introduction

The inquiry and utilization of minimal effort and sustainable materials have increased a prime significance from established researchers. Since, in the previous barely any many years, a lot of consideration has been paid to bone substitute materials designing. Cellulose is a promising material among inexhaustible assets. Cellulose is a sinewy, semi-translucent polymer of high molar mass and is the most plentiful biopolymer on around the world, which is inexhaustible, carbon-impartial, biodegradable, manageable, and cheap crude material. Cellulose can be gotten from not just wood and plants like cotton and green growth yet additionally a few animals, for example, microorganisms which are known to deliver it without anyone else. Cellulose is a direct polysaccharide made out of glucoses connected each other with β 1-4 glycosidic bond. Intermolecular hydrogen bonds interface hydroxyl gatherings and oxygen molecules in ring structure in glucose inflexibly and stable. These cellulose particles are accumulated along with van der Waals and intramolecular hydrogen bonds in equal and develop bigger microfibrils with 5-50 nm in breadth and a few micros long. This structure gives cellulose high hub durability[1,2,3-6].

Carboxymethyl cellulose (CMC) is a hydrophilic biocompatible polymer gotten through the substance adjustment of cellulose. It comprises of carboxymethyl bunch joined to the polysaccharide spine making it a polyelectrolyte. It has been recognized as a shrewd cellulose material as its properties, for example, shape, mechanical inflexibility and porosity can be

changed in a controlled way [1,32,33].

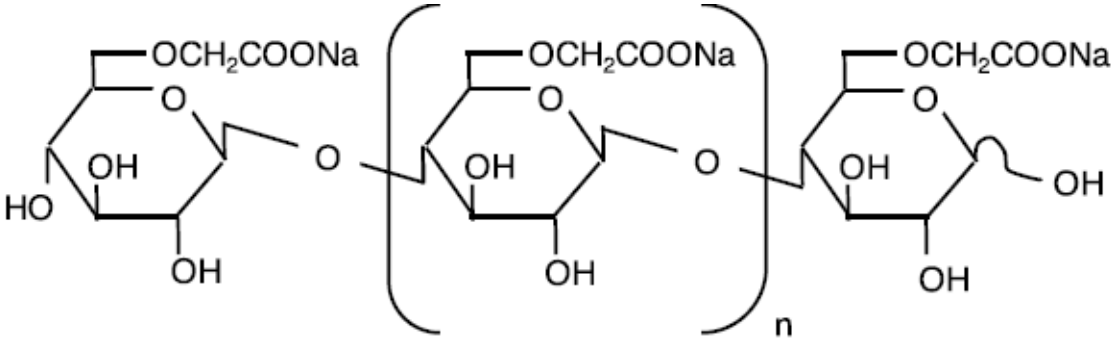

Fig. 1. The structure of sodium carboxymethyl cellulose (CMC).

The presence of carboxymethyl group in CMC makes it soluble in water and negatively charged polymer. This enables it to undergo complexation with oppositely charged materials thereby forming a crosslinked matrix structure with improved physico-chemical and biological properties. CMC has been studied as an injectable gels, composites and films for potential bone regeneration applications. In vivo study done with CMC showed that when used as a hybrid injectable material with calcium phosphate and bone morphogenetic protein can induce greater bone formation in rat tibial defect site.

Injectable scaffolds have been extensively studied in bone tissue engineering in the recent years mainly because of their potential to minimize the surgical interventions [10]. Scaffolds in the form of injectable gels offer advantages such as easy handling properties and adaptability to the defect site [24, 25, 34]. In order to make these injectable gels promising for bone tissue engineering, they should be able to incorporate drugs and bioactive agents and release them in a controlled manner [4]. Studies have shown that growth factors incorporated directly into gels showed the large initial burst release [5]. Particle (micro- and nano-) based injectable scaffolds can offer advantages in this aspect because of their ability to encapsulate the bioactive agents and release them in controlled manner [12, 13]. In addition, microparticle based scaffolds provide a temporary support for cells to attach and proliferate [27, 30].

Hydroxyapatite (HAP) which has good biocompatibility, bone conductibility is the main component of natural bone inorganic salt, and it has been regarded as an ideal material of bone defect repair [2]. Hydroxyapatite is a naturally occurring

mineral of biological and agricultural importance [8]. It is the major component and an essential ingredient of normal bone and teeth [26]. It is a naturally occurring form of calcium apatite with the formula $Ca_5(PO_4)_3(OH)$ but is usually written $Ca_{10}(PO_4)_6(OH)_2$ to denote that the crystal unit cell comprises two molecules [1]. Hydroxylapatite is the hydroxyl end member of the complex apatite group. The OH- ion can be replaced by fluoride, chloride or carbonate [11].

Theoretically, it is possible to combine several ions to form an apatite, but in practice only structures that are thermodynamically stable can be formed [14]. In particular, hydroxyapatite with the chemical formula $Ca_{10}(PO_4)_6(OH)_2$ the hexagonal crystalline structure is a combination of calcium phosphate, which forms the main body of the bone tooth tissue, shell and eggs [7]. The hydroxyapatite particles contained in the bone and teeth have, respectively, plate and pinion morphologies with nano dimensions [9, 16]. Since HAP is a main component of human's hard tissues, it obviously has a great biocompatibility and totally non-toxic. Due to these abilities, biomaterials made of HAP have been focused for a long time and many researches [17-20].

In this work we have synthesized and investigated the frequency dependence dielectric properties of Hydroxypatite and Carboxymethyl Cellulose (CMC) biopolymer based composite film at different filler concentrations with high frequency ranges. We focused on the dielectric constant, dielectric loss and conductivity of the composite film.

Experimental:

The HAP/CMC based nano-composites were prepared by solution casting method. HAP with its ceramic form(white powder), its chemical form $Ca_{10}(PO_4)_6(OH)_2$ density 3.18 g/cm^3, molecular weight 1004.69 g/mol, melting point 1670, refractive index 1.63. CMC its chemical form ($-CH_2-COOH$) is an anionic water soluble polymer; density 1.6gm/cm^3; molecular weight 250000 g/mol, variable melting point 274^0C. All above are raw materials. We have synthesized HAP/CMC polymer composite thin film for five concentration such as [20:80] weight fraction of (HAP:CMC) in our laboratory. At first, we have synthesized thin film for 20:80 concentration. First of all, we have taken 1.6gm CMC and 0.4gm HAP. Then, we have taken 20ml distilled or deionized water in a beaker. Then we heat the water at 60^oC at the oven. As CMC is not proper water solvent, we warm up the water for

which the CMC dissolved properly in water. Then we stirred this solution by magnetic stirrer for 30 minutes. The resulting solution will be clear and strongly viscous. When CMC is totally dissolved in water, then we have added 0.4gm HAP to this solution and again continuously stirred this solution by magnetic stirrer at high temperature for 1-$1^1/2$ hour. When the solution is evaporated them this solution is turned into the form of gel. Next we have poured this gel in a petri-dish and it is kept for 48hrs at room temperature. Finally we have prepared HAP/CMC composite thin film for this 20:80 thin film at room temperature.

Results and discussions:

The interpretation of the XRD pattern obtained of film shows limited crystallinity due to hydrogen bond interaction among the hydroxyl groups present in polymer chain. The broad diffractions occurred at $2\boldsymbol{\theta}$ angles ($\sim 26^o, 33.5^o, 34^o, 35^o$). Three broad humps 25 and 35 indicate the semi-crystalline nature of HAP which contains both the crystalline and amorphous region. The XRD patterns show that the peak intensities and widths were varied with the increasing concentration of CMC. This indicated that the ratio between hexagonal lattice and primitive lattice is changed when the dosage of CMC changed.

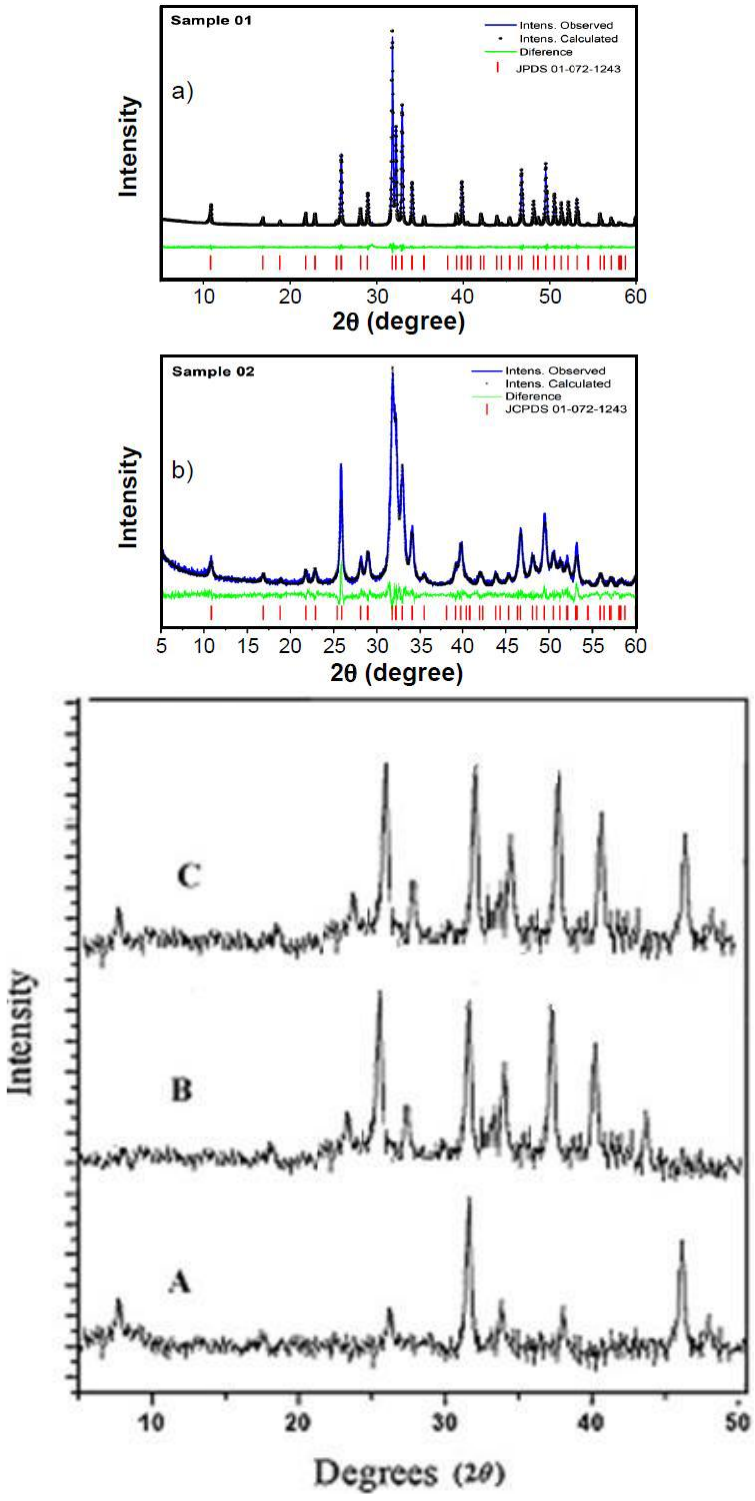

Fig 2: XRD spectra of (A) CMC,(B) HAp and(C) n-HApCMC

The morphology and particle size of the as-obtained HAP nanocrystal are also directly characterized by SEM (Fig. 3). It can be seen that micro-sized hydroxyapatite particles piled together like plate were obtained when the Ca^{2+} and PO_4^{-3} solution without CMC used to synthesis submicron HAP. This implies

that the structure and the particle size of HAP can be easy controlled by changing the amount of CMC used in the synthesis [21, 28, 29, 31].

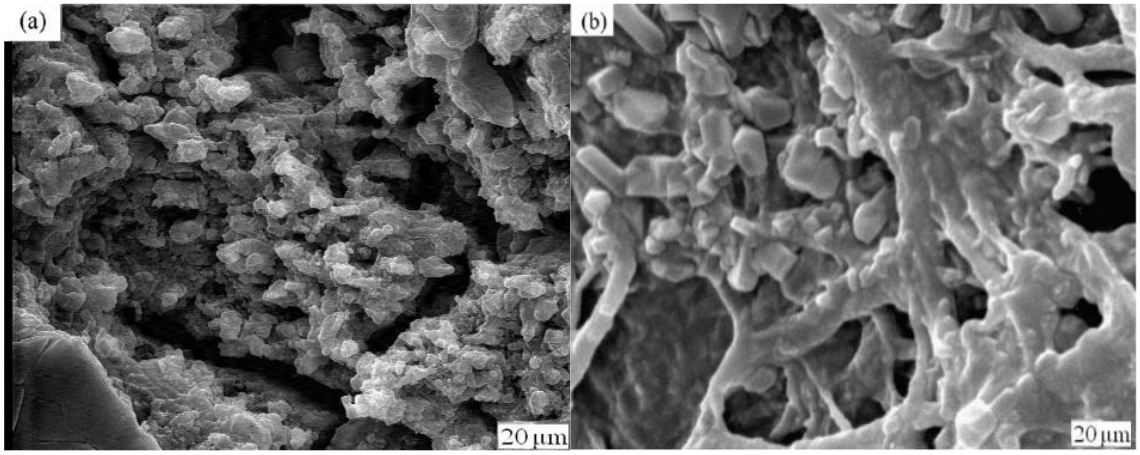

Fig 3: Scanning Electron Microscope of HAP and CMC

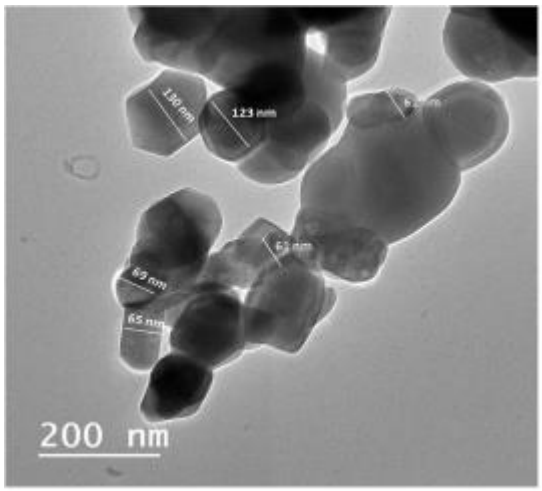

Fig. 4: TEM image of the HA powder calcined at 900°C for 2h.

Particle size analysis (TEM)

The TEM observation of the as prepared HA powder calcined at 900°C for 2h., Figure (4); revealed that the particles have a crystalline size in the nano-scale ranging between 61 to 130 nm.

FT-IR spectra of functionalized pure HAP/CMC blends in weight fraction showed characteristic band of bending and stretching vibrations of the functional groups formed in the prepared film [3, 15]. FT-IR absorption band positions and their

assignment of the prepared sample describe here. For functionalized HAP/CMC the broad peak at 3427.51cm^{-1} is due to (OH) stretching vibration of hydroxyl groups and peak at 1720.64cm^{-1} represent the stretching vibration of C=O stretching from the carboxylic groups(-COOH). The band at 3570.24cm^{-1} was assigned to CH_2 asymmetric stretching vibration. The band at about 603.72cm^{-1} corresponds to C-O stretching of carbonyl groups.

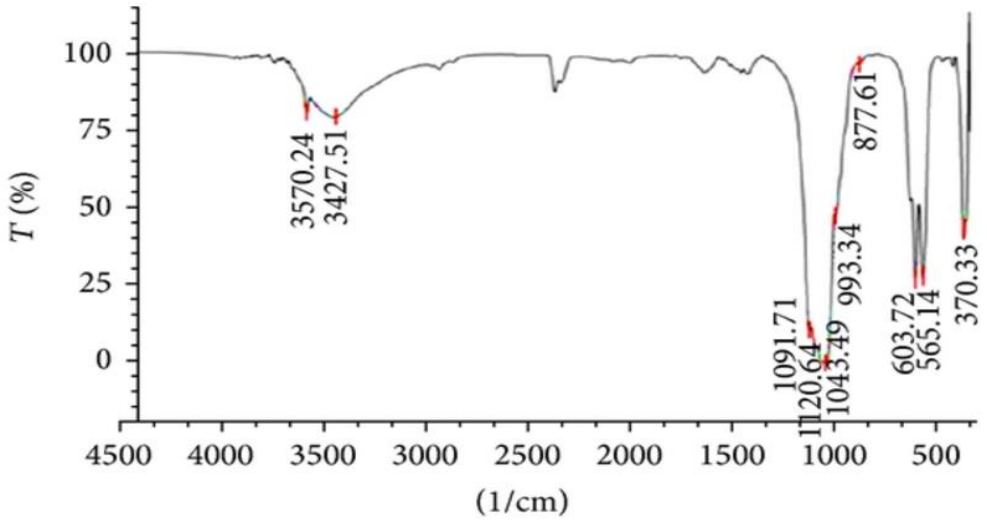

Fig 5: FT-IR spectrum of HAP/CMC

Dielectric properties measurements were done in the frequency range from 40Hz to 200kHz at room temperature. Dielectric and impedance spectroscopy measurements system is used to know dielectric behaviour of the materials.

The Fig. 6 shows the relation between Dielectric Constant and Frequency at room Temperature. At low frequency the dielectric constant of HAP/CMC composite film is high and when the frequency increased the dielectric constant decrease. This decrease is due to the reduction of space charge polarization effect.

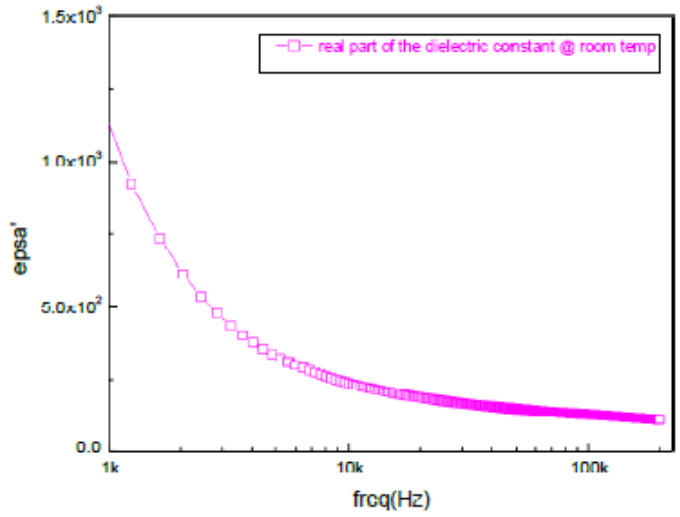

Fig. 6: Real part of the Dielectric constant vs. Frequency at room temperature

The Fig. 7. shows the relation between AC Conductivity and Frequency at room temperature. It shows that at low frequency conductivity of the material is low and at high frequency conductivity is high. The conductivity increases with increasing frequency due to possible release of space charge.

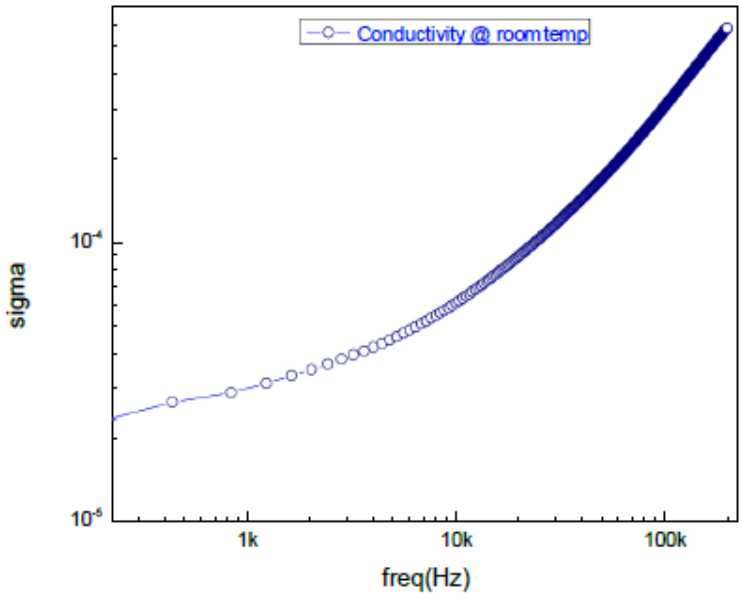

Fig. 7: Conductivity vs. Frequency at Room Temperature

The complex manner of variation of dielectric loss of HAP/CMC composite film with frequency at room temperature is shown in Fig.8.

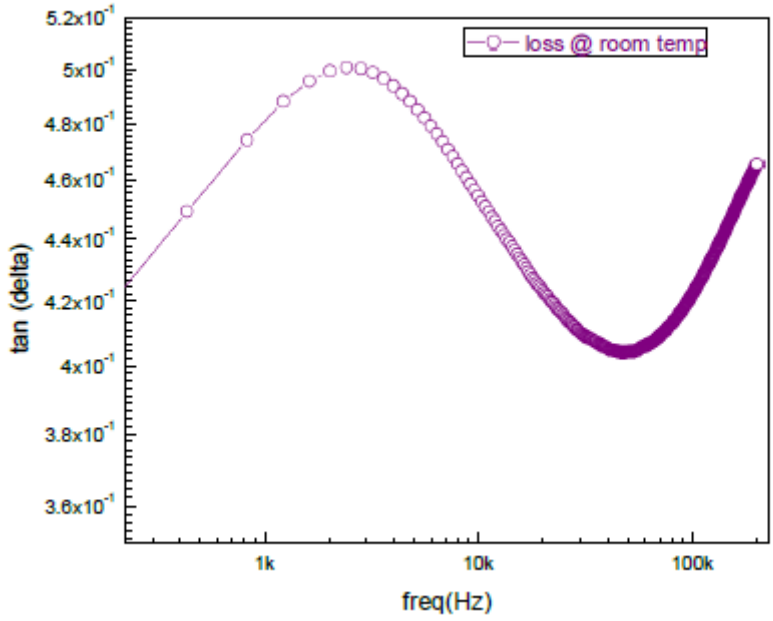

Fig. 8 tan δ vs. Frequency at room temperature

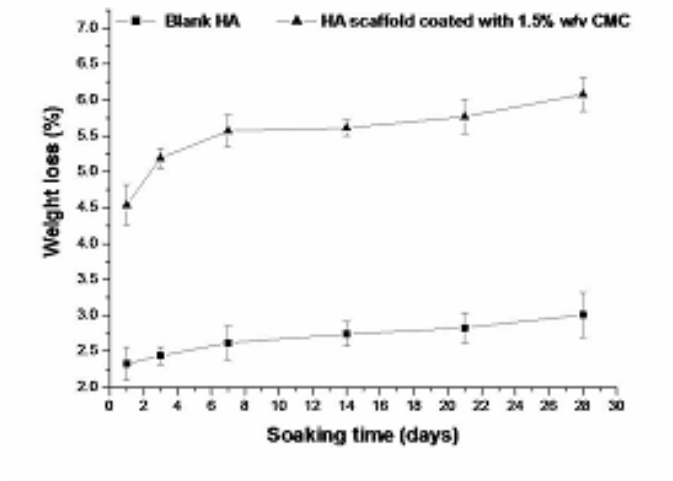

Fig. 9: The total weight change of the pure HA and 1.5% w/v CMC/HA porous scaffolds as a function of immersing time in SBF solution at 37°C.

Biodegradability:

Biodegradability of the 3D organic/inorganic scaffold has an important role in biomedical applications. Scaffolds should be degradable naturally over the time to permit new tissues to grow. Since CMC is a water-soluble polymer, we carried out in vitro degradation test to determine the weight loss of the developed composite scaffolds [6]. Figure (9) represents the weight change of pure HA and HA scaffold coated with 1.5% w/v CMC as a function of immersing time in stimulated body fluid (SBF) solution at 37°C [23]. The figure shows that the rate of weight loss increases from 4.54% in the first day to 6.08 in the 28th day. It is noticeable that the 1.5% w/v CMC composites scaffolds have higher weight loss 6.08% in comparison to HA scaffold samples without coating, 3.01%. It is due to the presence of the cross-linking of the carboxymethyl, which is soluble in water (Jiang *et al*., 2009; Lu *et al*., 2007).

Conclusions:

Polymer composites based on HAP as a matrix and CMC as reinforcement were successfully prepared using solution casting method. Noticeably, the controlled structure HAP was easily synthesized assisted by CMC when the synthesis is performed at room temperature. The physical and chemical performance of the as-synthesized composite film totally meet the requirements of bone tissue engineering material. The AC conductivity, dielectric permittivity and loss is a function of frequency and [HAP:CMC] concentration ratio. Recycled eggshell was efficiently used by low-cost, eco-friendly methods to fabricate 3D porous scaffolds. The biodegradability test indicates that the scaffold composites are degradable naturally over the time.

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