The Physical Characteristics of Composite Bone Scaffold Fabricated from OPEFB-CMC, Chitosan and HA

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***Abstract*—Oil Palm is the largest agriculture in Malaysia and amongst the world. Unfortunately, difficulty occurs in which oil palm disposals lead to unpleasant contamination and bad scenery. However the combination of oil palm empty fruit bunch – carboxymethyl cellulose (CMC), Chitosan (CS) and hydroxylapatite (HA), turns the composite into biodegradable bone graft as known as porous composite. It was produced with lots of pores through co-solution and lyophilization method. Pores process is important for tissue engineering development but a little bit crucial to achieve ideal properties. An ideal bone scaffold should contain more pores for better cell proliferation and as nutrient carrier. Unfortunately, too many pores may lead to the weaknesses of the scaffold. The research had examined samples through compression tests for strength. It turns out that OPEFB-CMC, a derivation material from EFB, together with HA and CS had an increment in their toughness compared to previous research using only HA and CS. This paper intentionally presents detailed evaluation about bone scaffold fabricated from waste product as the polymer, with HA and CS for better biodegradable bone implant.**

***Index Terms*—oil palm empty fruit bunch, bone scaffold, mechanical test, pore size.**

I. Introduction

Half of the world's palm oil production is produced in Malaysia in about 10.8 million tonnes [1]. As a result, it generates a large quantity of solid wastes including oil palm trunks (OPT), oil palm fronds (OPF), empty fruit bunches (EFB) and palm pressed fibres (PPF), as mentioned earlier from previous work [2]. Table 1 summarizes the types of waste or renewable energy resources in Malaysia with rough estimation in Ringgit [3].

EFB for example is a resource with huge potential to be used in power generation and surprisingly, this waste could be used in biomaterial usage. To narrow the work, it is best suited in biodegradable bone implant. The derivatives from EFB that are more prominent and frequently used are cellulose acetate (CA), cellulose phosphate (CP) and carboxymethyl cellulose (CMC) [4]. It entices researchers to utilize these sources in any field.

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TABLE I. Types of Wastes in Malaysia and its Energy Value [3]

Renewable energy source	Energy value in RM million (annual)
Forest residues	11,984
Oil palm biomass	6379
Solar thermal	3023
Mill residues	836
Hydro	506
Solar PV	378
Municipal waste	190
Rice husk	77
Landfill gas	4

Among these three substances, CMC was chosen as the biopolymer ocupied in biodegradable bone implant. Considering the said reason, CMC is the only material that is able to biodegrade by itself compared to CP and CA. It reaches the set standard that requires the bone implant to biodegrade itself during bone self-healing duration.

II. Methodology

A. OPEFB-CMC Preparation

The fabrication of the sample began with the preparation of CMC, synthesized from EFB. The procedures were:

i. Pre-hydrolysis process – an essential process needed to remove hemicellulose

ii. Soda pulping – to convert fibre of EFB into pulp by mixing it with Sodium Hydroxide (NaOH)

iii. Oxygen-Ozone-Peroxide bleaching processes – to clean up the EFB pulp and turn the color into white without using any chlorine product

iv. Carboxymethylation of OZP pulp – Convert OZP pulp into OPEFB-CMC. Final appearance of the product is white powder

B. Scaffold Preparation

The sequenced steps in producing porous bone scaffold:

i. Dissolve OPEFB-CMC in distilled water by sonication technique for 10 minutes.

ii. Add 4g HA and 3g Chitosan gradually into CMC solution.

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iii. Agitate the mixture solution evenly under constant stirring at room temperature for 2 hours.

iv. Add 3ml glacial acetic acid into the mixture solution to obtain slurry mixture.

v. Then, pour the slurry mixture into mold with desired shape and keep in refrigerator for 24 hours to be frozen.

vi. Finally, put the frozen samples in freeze-dryer for 3 days for lyophilization process to obtain a lot of pores in the scaffold.

C. Sample Testing and Analyzing

All the samples underwent several analyses and testings. There were compression test for mechanical testing and also SEM analysis to obtain the pore sizes contained in the scaffold.

For compression test, the standard used was ASTM D 695 which covered the mechanical properties of unreinforced and reinforced rigid plastics, including high-modulus composites, when loaded in compression at relatively low uniform rates of straining or loading. The standard speed of testing was done at 1.3 ± 0.3 mm (0.050 ±0.010 in.)/min. The dimension for the samples is shown in Fig. 1.

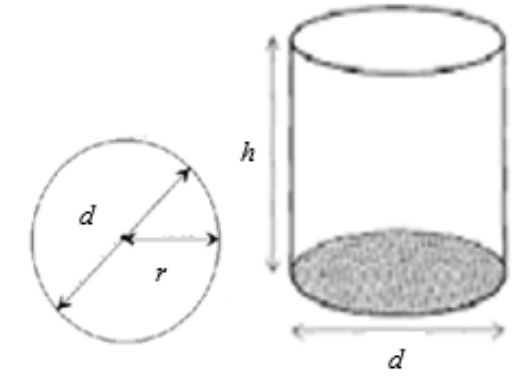

Figure 1. The standard dimension for test specimen

For SEM analysis, a very thin layer of the scaffold was mounted on an aluminum stub and was sputter-coated with platinum prior to morphological examination.

III. Results and Discussions

A. Mechanical Test: Compression

The percentage of CMC began with 30%, 40% and 50%. It was for example, labeled as B30, B40 and B50, respectively. Those percentages of CMC were chosen for preparing bone scaffold because there was almost no strength when CMC percentage was below 30% and CMC started to lose their strength after 40% concentration.

TABLE II. Porous Bone Scaffold Labeling and Compressive Strength

CMC concentration (%)	Materials ratio	Compressive strength (kPa)
B:	Ratio (CMC_C: HA + CS) (%)	
30	30: 50	32
40	40: 50	50
50	50: 50	42
D:	Ratio (CMC_C: nHA + CS) (%)	
30	30: 50	30
40	40: 50	42
50	50: 50	40
E:	Ratio (CMC_A: HA + CS) (%)	
30	30: 50	24
40	40: 50	4
50	50: 50	8
F:	Ratio (CMC_A: nHA + CS) (%)	
30	30: 50	12
40	40: 50	16
50	50: 50	4

By referring to Table II, the compressive value for the composite scaffold was low if compared to range of cancellous bone (2000 ~ 10, 000 kPa) [5] but still higher compared to previous research (0.0024 – 2 kPa) [6]. The probability of lower compressive strength value probably caused by the fabrication method or higher porosity content of the scaffold that decreased the strength of the composite.

Table III shows an example from previous research that used chitosan as its base material in fabricating bone scaffold combined with calcium phosphate as its bioactive material. The compression strength recorded for chitosan scaffold in different medium was in range 0.0042 – 0.72 kPa. Meanwhile, some improvement was added to the scaffold with the addition of calcium phosphate, which has increased its strength and recorded in range of 0.0024 – 2 kPa.

TABLE III. Compressive Modulus of the Chitosan Pure and Chitosan/Calcium Phosphate Composite Scaffold [6]

Solution	Compressive modulus of the scaffold (kPa)	
	Chitosan scaffold	Chitosan/HAp composite scaffold
Non-solvent	0.72	2.00
Sterilization solvent	0.07	0.26
Cell culture media	0.0042	0.0024

Fig. 2 demonstrated compression strength versus CMC of different concentrations were obtained for samples B, D, E and F. Transparently from the graph plotted, sample B shows higher value of compressive strength with the highest reading at B40 with value of 50 kPa. Followed by samples D, E and F with their optimum values noted at D40 with 42 kPa, E30 (24 kPa) and F40 with their values

of compressive strength were calculated at 16 kPa, respectively.

The compression test also indicated that at this optimum percentage, it showed lower strength at 30% CMC compared to 40% because the composite lacked CMC polymer for samples B, D and F. There was a bit of a difference for sample E since its optimum strength was observed when CMC was at 30% concentration. This could be due to uniformity of CMC during stirring with chitosan and HA. In the meantime, at 50% CMC, the polymer content had excess which led to undermining of composite strength.

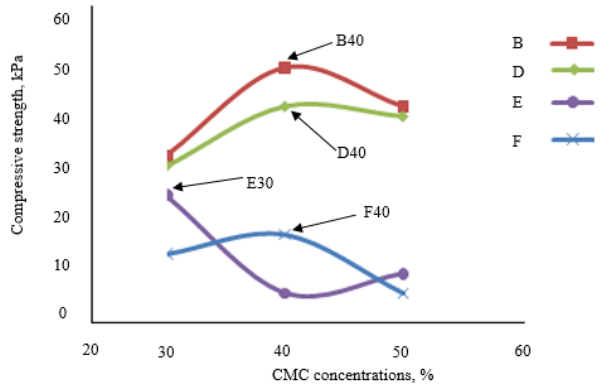

Figure 2: Compressive strength versus CMC concentration of scaffold for (a) Sample B, (b) Sample D, (c) Sample E and (d) Sample F

B. SEM Analysis

An open, fully interconnected pore for scaffold is necessary for providing a large surface area. That attribute allows cell ingrowth, uniform cell distribution and facilitates vascularization of the construction. Besides that, pore size is also a critical issue because occlusion by the cells will happen if the pores produced are too small. This would prevent cellular penetration resulting in failure of extracellular production.

Pore size for B40 composite scaffold at the surface varied from 30–60 µm and 150–170 µm whereas the size at the cross section was measured in range of 130–150 µm and 400 µm. D40 size measured on its surface was 20 and between 80–100 µm and varied from 90–100 µm size traced at the cross section. While that, E30 had sizes between 20–50 µm and 70–100 µm measured at its surface and ranged between 100–120 µm, recorded at its cross section. For F40, the pore size measured on the surface was 20 µm and between 80–100 µm. While that the pore size at the cross section was measured from 100–130 µm. All the measurements were taken from SEM images as displayed in Fig. 3.

Based on previous references [7, 8], large pore sizes between 100–150 µm and 150–200 µm were substantial for bone ingrowth. Smaller sizes between 75–100 µm resulted in ingrowth of unmineralized osteoid tissue [9]. Osteoid tissue is a type of organic portion of the bone matrix that forms prior to the maturation of bone tissue (young bone that has not undergone calcification). Smaller pores between 10–44 µm and 44–75 µm were suitable for penetration only by fibrous tissue. Fibrous tissue is composed from bundles of collagenous white fibres. A study conducted previously [10], have demonstrated that chondrocytes showed preferential proliferation and extracellular matrix (ECM) production for scaffolds with pore sizes between 250 and 500 µm. This was because, as supported by study from Whang, Healy & Elenz (1999), the new bone might be grown on a surface (osteoconduction) since osteogenesis, defined as the formation of bone, was induced [11].

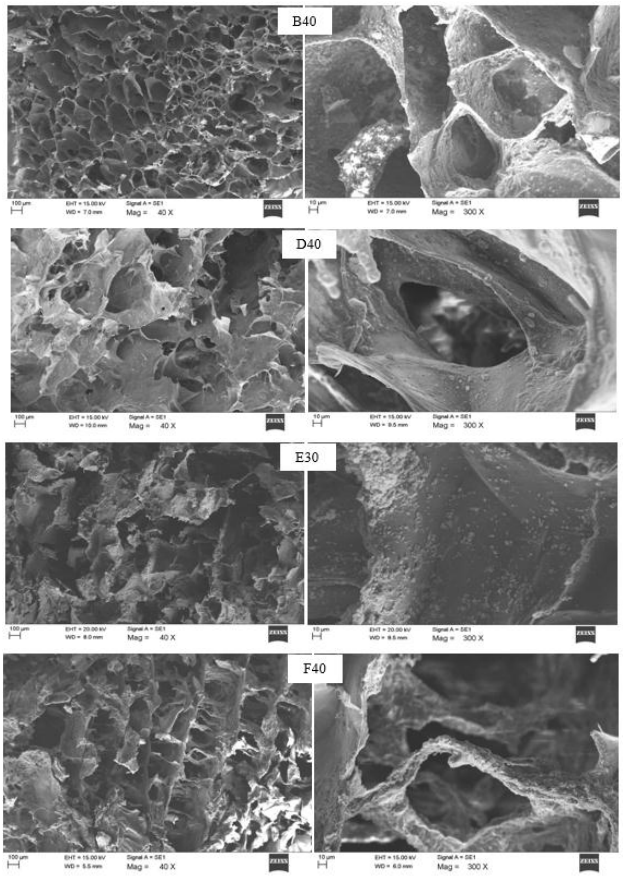

Figure 3. SEM images for B40, D40, E30 and F40. Figures shown are at magnification of 40 times (left), and at magnification of 300 times (right)

Previous researcher has mentioned that the minimum pore size required to regenerate mineralized bone was generally considered to be at about 100 µm [8]. Relatively larger pores favor direct osteogenesis, since they allow vascularization and high oxygenation, while smaller pores result in osteochondral ossification, although the type of bone ingrowth depends on the biomaterial and the geometry of the pores. However, in the case of non-loading bearing condition, bone ingrowth was similar in all pore sizes suggesting that 100 µm may not be the critical pore size [12]. Table 4 has simplified suitable tasks carried out due to pore size for each type of composite scaffold.

TABLE IV. PORE SIZE WITH SUITABLE TASK ALLOCATION FOR FOUR TYPES OF SCAFFOLDS

Type of scaffold	Pore size (μm)		Tasks allocation
	Surface	Cross section	
B40	30–60, 150–170	130–150, 400	Substantial for bone ingrowth Penetration by fibrous tissue only Suitable for chondrocytes proliferation ECM production for scaffolds
D40	20, 80–100	90–100	Substantial for bone ingrowth Suitable for ingrowth of unmineralized osteoid tissue Penetration by fibrous tissue only
E30	20–50, 70–100	100–120	Substantial for bone ingrowth Suitable for ingrowth of unmineralized osteoid tissue Penetration by fibrous tissue only
F40	20, 80–100	100–130	Substantial for bone ingrowth Suitable for ingrowth of unmineralized osteoid tissue Penetration by fibrous tissue only

In general, all composites fabricated in this work were suitable for bone ingrowth because each of them had minimum allowable pore size for regeneration of mineralized bone. The close-up images as exhibited in the right column of Fig. 3 show that all scaffolds possess interconnected pores. It means that each pore is connected to one another to ensure that blood is supplied in order to provide for mass transfer of oxygen and nutrients [7]. It is also to ensure the survival of the implant to act with positive development when surrounded by human liquid inside the body.

The morphology of all specimens has significantly displayed an open pore structure with both micropores and macropores. These types of pores are good for blood supply and cell attachment. The created pore architectures produced by freeze drying technique with B40 composite scaffold in this research was the most similar pattern among the other three samples if compared to previous publication as shown in Fig. 4. More pores were traced at the surface of B40 scaffold as shown in Fig. 4 (a) compared to chitosan/gelatin in Fig. 4 (b). This could be due to the excessive natural polymer in B40 contributed from chitosan and CMC whereas the other scaffold has less pores because the polymer contribution was only from chitosan. Moreover, B40 provided more sizes for pores created that lead to more diverse functionality.

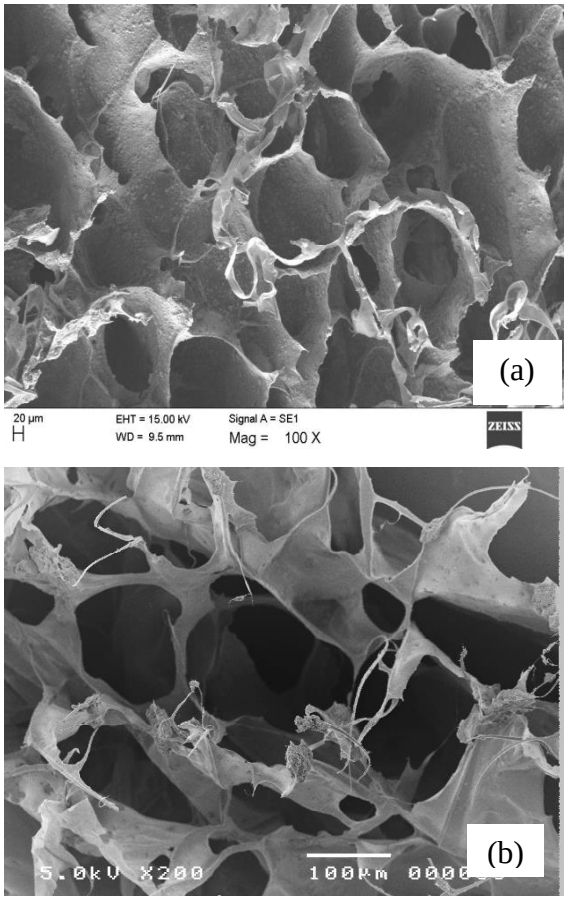

Figure 4. Porous scaffold composite with an open pore structure: (a) B40 composite scaffold and (b) chitosan/gelatin composite scaffold [13]

IV . CONCLUSION

A tough biocompatible composite scaffold constructed through a co-solution method and freeze-drying approach for pore textures has been successfully developed. CMC is found to be a suitable material as a reinforcement element to cope with chitosan and HA/nHA producing better composite which is applied in biomaterials for orthopaedic application as bone scaffold. It is proven that the material is able to hold together and improve interaction between chitosan and HA/nHA. This material could disperse the HA/nHA more uniformly and provide stronger compressive strength when compared to composite scaffold which was only fabricated from chitosan and HA/nHA. Compressive mechanical tests on the scaffolds showed that the composite scaffolds had compressive elastic moduli in the range of 4–40 kPa, pretty much higher compared to chitosan and HA/nHA composite that were recorded in the range 0.002-0.0024 kPa only and appeared not to be affected by the high porosity of the scaffolds. Thus, the composite scaffolds containing chitosan and HA/nHA reinforced with CMC were observed to be biocompatible. Porosity and pore size, both at the macroscopic and the microscopic level, are important morphological properties of biomaterial scaffold for bone regeneration. High porosity and large pores enhance bone ingrowth and osseointegration of the

implant after surgery. In this research, B40 possessed the highest porosity percentage which was 94.9%. This feature could promise positive development of new bone formation. The scaffold has also been measured to have the largest size of pores compared to others with the biggest value being 400 μm and it is the answer to why it has higher swelling ability of 92.87%.

There is, however, an upper limit in porosity and pore size set by constraints associated with mechanical properties. An increase in the void volume results in a reduction in mechanical strength of the scaffold, which can be critical for regeneration in load-bearing bones. The extent to which pore size can be increased while maintaining mechanical requirements is dependent on many factors including the nature of the biomaterial and the processing conditions used in its fabrication into 3D scaffolds. An upper limit is also set from the dimensions of the pores of the specific bone-tissue repaired. Because of it, in this work, the highly porosity gives higher value in compressive strength. It is concluded that the interaction bonding created between the molecules are really strong.

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