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Review on Eggshell Waste in Tissue Engineering Application

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Abstract: Eggshell waste (EW) is an abundant waste from group of municipal solid waste that being neglected for a long time until its potential in numerous field being developed. High calcium content in EW make it favorable as potential starting material to be mixed with other biomaterial for tissue engineering application. This paper reviews the utilization of eggshell in producing calcium phosphate and particular attention has been given to hydroxyapatite because of its bioactivity and osteoconductivity and tricalcium phosphate because of its resorbability in physiological medium. This reviews reveals interesting information and developments that have not, to our knowledge, previously been reported. Starting with the eggshell unique structure and properties with its application in various field as promising biomaterials. In addition to that, the emerging advanced manufacturing makes it possible for incorporating EW in biocomposite including metallic, ceramic and polymer. Similar to synthetic composite, this system also strive for excellent performance with exceptional in mimicking the structure of living materials along with providing biocompatibility.

Keywords: Eggshell waste , calcium phosphate , hydroxyapatite , tricalcium phosphate , biocomposite

1. Introduction

Waste production come from various way such as household, agricultural and industrial. Based on percentage, municipal solid waste contribute the largest percentage of waste that is 64% followed by industrial waste (25%), commercial waste (8%) and 3% consists of construction waste in Malaysia [1]. The factors influence the composition and characteristics of municipal solid waste are economic level, season, weather and culture of people living or doing business in the area the waste generation [2]. Based on Dr Mohd Pauze Mohamad Taha, Solid Waste Management and Public Cleansing Corporation (SWCorp) deputy chief, about 38 142 tons per day waste being produced in Malaysia and surprisingly all this waste being dump while the percentage of it availability to be recycle up to 40% [3]. Based on globally statistic, 2.01 billion tons waste being produced per year and will rise up to 3.4 billion tons per year in 2050 due to urbanization and growing population [4]. Municipal solid waste still leading the percentage of waste that being dump in globally that it 64 % from all type of waste. In order to decrease statistic of waste that being dump, recycle the waste material seems the most suitable way to start. Possibility to make limitation for a person to throw only specific weight of waste seems impossible as waste that being produced could not be control. With recycling the material that have potential to be used again or to become new material, it may help our Earth from being filled with dumping waste. Most researcher try to develop new material from dumping waste in order to help decreasing the total waste that being produced and mostly shown the successful result in their research. One of the waste that have potential to be develop is eggshell waste (EW). Based on statistic, 250 000 tons of EW is produced annually worldwide and currently cumulated on-site without any pre-treatment [5]. In Malaysia about 642,600 tons of egg is produced every year and from this total about 70 686 tons of EW is expected to be generated every year and being dump to landfill [6]. Production of eggs increase every year due to high demand in food industry such as cake, dish and also in agricultural industry due to high

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demanding in chicken and birds meat. This is the reason why researcher interested to use EW as new material due to it continuous source.

1.1 Formation, Function and Structure of Eggshell

Formation of eggshells occur in uterus where it mineralized rapidly 10 to 22h after ovulation in uterus. The uterus or shell gland histologically consists of pseudo-stratified ciliated columnar epithelial cells, which include ciliated and non-ciliated cells, basal cells, and goblet cells [7]. Eggshell significance is related to its function to resist physical and pathogenic challenges from the external environment, and addition to providing a source of nutrients, primarily calcium, for embryo development [8]. Basically, an eggshell is made up by a three layered structure, namely the cuticle, the spongeous layer and the lamellar layer that support it function as protector and nutrient storage for embryonic respiratory component [9]. Fig. 1 below show the illustration of eggshell structure.

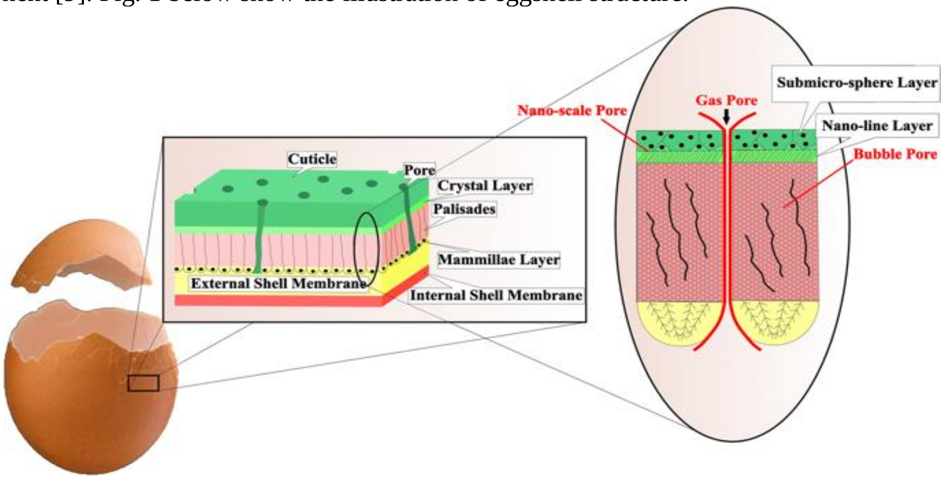

Fig. 1 - Illustration of eggshell structure [10]

Based on Fig. 1, it can be seen that from inside outward eggshell have several layer that are membrane, mammillary layer, palisades, crystal layer and cuticle layer: Membranes are a fibrous structure situated between the eggshell and egg albumen. It semi-permeable membrane form from composed of protein fibres that are arranged to become it form. They are essential for the formation of the eggshell and also provide the shell foundation except at the blunt pole of the egg where they separate to form the air-space [11].The inner membrane remains uncalcified, while the fibres of the outer shell membrane become mineralized at discrete sites and become incorporated into the base of the eggshell [8]. Mammilary layer made up from attraction of calcium salt by outer surface of the outer shell membrane layer in that region of the oviduct termed the tubular shell gland. Mammilary layer is the main source for Ca mobilization during embryonic development [12].

Palisades is the thickest aspect of the shell comprises about 200 µm thickness. Each palisade column grows from one mammillary knob and as the calcification mechanism proceeds adjacent columns fuse that function to resist exterior compressive forces, while permitting chick pipping from the inside by pushing outwards on the narrow cone bases at the time of chick hatching [13]. Palisade columns grow from one mammillary knob and as the calcification process proceeds, adjacent columns fuse. This layer ends at the vertical layer which has a crystalline structure of higher density than that of the palisade layer [8]. Crystalline layer of the shell responsible for its mechanical strength, consists of more than 90% calcium in the form of calcium carbonate [14]. Cuticle is the most outer shell in eggshell structure and an uneven organic layer covering the outer surface of the eggshell [15]. It made up from glycoproteins, polysaccharides, lipids and inorganic phosphorus. Prior to the formation of the cuticle, the shell is overlain by a vertical crystal layer in which the protein moiety is also vertically orientated [16]. Cuticle function to governing water exchange by deflecting water or preventing its loss, and may function in limiting microbial colonization of the eggshell surface. The cuticle constituents also plug the eggshell pores and thus physically limit bacterial entry [17].

1.2 Eggshell Content, Properties and Application

All layer have different function and related to each other. The shell structure might have a significant effect on eggshell characteristics, mainly thickness and strength [18]. This is the reason why eggshell really important in egg structure. According to composition of eggshell, it contain calcium carbonate (calcite), $CaCO_3$ as main component in eggshells and is the major inorganic substance found in an egg. Calcium in eggshell function as source of developing embryo [19]. That is the reason why calcium contain in eggshell high. Others are organic matter which magnesium carbonate ($MgCO_3$) and calcium phosphate (CaP) as well as insoluble proteins [20]. Some researcher have investigate $CaCO_3$ content from different source of eggshell waste and it being summarized in Table 1 below.

Table 1 - Various types of EW with it $CaCO_3$ content

Researcher	Type of EW	$CaCO_3$ content
Ajayan N. et al, 2020 [21]	Duck EW	91%
Shwetha A. et al, 2018 [14]	Pigeon EW	91%
Shwetha A. et al, 2018 [14]	Rat Snake EW	81%
Shwetha A. et al, 2018 [14]	Swift EW	68.06%
Marchal R. L. S. B. et al, 2017 [22]	Ostrich EW	97%
Tangboriboon N. et al, 2016 [23]	Quail EW	97.45%
Neunzehn J. et al, 2015 [24]	Chicken EW	93.6%
Al-Obaidi F. A. et al, 2012 [25]	Sparrow EW	95%
El-Ishaq A. & Kida H.D., 2011 [26]	Guinea fowl EW	98.7%

Table 1, shows that various source of EW can be found in the world. Although EW like rat snake, pigeon, sparrow and swift not contribute so much percentage in total of waste that being produced, several researcher still investigated about it in order to show that most of the EW that produced contain $CaCO_3$ as main composition in eggshell. Different percentage of $CaCO_3$ in different type of animal occur due to its habitat and diet [14]. Food that being consumed by animal may affect the percentage of $CaCO_3$ contain in eggshell that being produced and also it strength [27]. Mechanical properties of quail, goose, chicken and ostrich had being investigate by Hahn E. N. 2017. In his study, chicken eggshell shown highest strength with ratio 28.8 MPa for all type of chicken eggshell followed by quail (25.6 MPa), goose (17.9 MPa) and ostrich (8.5 MPa). This result affected by protein content in evolution of avian eggs that relate to animal diet as mentioned before [28]. Table 2 below shows application of EW in various field.

Table 2 - Application of EW in various field

Application field	Researcher
Biodiesel catalyst	• Bharadwaj A. V. S. L. S. et al, 2019 [29] • Peng Y. P. et al, 2018 [30] • Asri N. P. et al, 2017 [31] • Ngadi N. et al, 2016 [32] • Tan Y. H. et al, 2015 [33]
Adsorbent in waste water or soil	• Makuchowska-Fryc J., 2019 [34] • Tizo M. S. et al, 2018 [35] • Borhade A. V. & Kale A. S., 2017 [36] • Ali Z. T. A. et al, 2016 [37] • Pettinato M. et al, 2015 [38]
Fertilizer	• Andhare A. A. et al, 2019 [39] • Khairnar M. D. & Nair S. S., 2019 [40] • Radha T. & Karthikeyan G., 2019 [41] • Wijaya V. T. & Teo, S. S., 2019 [42] • Gaonkar M. & Chakraborty A. P., 2016 [43]
Biomaterial application	• Horta M. et al, 2019 [44] • Sayed M. et al, 2018 [45] • Demir D. et al, 2017 [46] • Ferreira J. R. M. et al, 2016 [47] • Biscaia S. I. et al, 2015 [48]

Table 2 shows application of EW in various field by different researcher. EW being used in biodiesel field as heterogeneous catalyst as processing of EW can produced calcium oxide (CaO) catalyst [29]. CaO is based catalyst that being choose by researcher as it need less reaction time and low temperature than acidic catalyst. However, most alkaline catalyst is costing, so extraction of CaO from EW being choose as alternative to substitute synthetic alkaline catalyst [31]. For adsorbent in waste water and soil, main content in EW that is $CaCO_3$ become popular among researcher because it can remove heavy metal from solution. $CaCO_3$ in EW can be used to replace milestone as milestone is nonrenewable resources [34]. Besides, high cost in using activated carbon can be solved by using EW as it is one of the abundant waste in landfill [35]. High calcium content in EW have attract researcher to use it as fertilizer due to its low cost than chemical fertilizer and it continuous source [39]. Use of chemical fertilizer can cause soil impurities in soil, so calcium needed to help reduces soil acidity which indirectly improves crop production where EW is one of the source with high calcium content. Lastly, EW also being used in biomedical application especially in bone tissue engineering area as calcium content in EW can be convert to CaP where CaP is main mineral in bone [45].

Development of CaP from EW is one of the way to overcome the limited source of bone tissue and to decrease the cost in implant surgery [44].

1.3 Biomaterials in Bone Tissue Engineering

In various type of EW application, biomaterial application of EW become trending among researcher due to it similarity with human bone characteristic. Calcium contain in EW suitable for bone scaffold after it being synthesize to new material that needed in bone tissue engineering application. Bone tissue engineering is based on the consideration of bone structure, bone mechanics, and formation of tissue as it aims to induce new functional bone tissues [49]. In other words, to successfully regenerate or repair bone, knowledge of the bone biology and its development is quite necessary [50]. In bone tissue engineering, biomaterials is chosen due to it biocompatibility where biomaterial able to perform its desired function while it function to body is appropriate and the ability to response in specifics situation [51]. Biomaterials is any substances or its combination, other than drugs, man-made or natural resource that can be used for any period of time which enhance or partially or totally any organ, tissue or body function to maintain or increase the quality life of individual [52].

Biomaterials can be divide into three types based on its application; metal, ceramic, and polymer [53]: Metal or metallic biomaterials is engineered systems designed to provide internal support to biological tissues using metallic material such as titanium and stainless steel [54]. Ceramic or bioceramic class of materials that is used for repairing or replacing damaged bone tissues using ceramic material such as calcium phosphate and alumina [55]. Polymer or biopolymer are chain-like molecules made up of repeating chemical units produced from renewable resources that able to degraded in the environment [56].

2. Eggshell Waste Reinforce for Bio composite

Materials are the basic elements of all natural and man-made structures. Metaphorically speaking, this materializes the structural conception. Technological progress is associated with continuous improvement of existing material properties as well as the expansion of structural material classes and types. In composites, materials are combined to make better use of their virtues with minimizing their deficiencies [57]. This process of optimization including aiming in cheaper cost can provide an alternatives to a designer from the constraints associated with the selection and manufacture of conventional materials. Most of designers are looking for materials having a superior mechanical properties without having a great weight [58]. The future development of composites (and its materials) to serve in healthcare industry have successfully attracted researchers into designing implant or scaffolds that aid in the healing process of bone traumas including fractures, tumors and infections [59]. Recent findings demonstrate that rate of degradation of composite materials can be controlled resulting an improved properties for tissue engineering applications [60]. As it is known, bone reconstruction processes and biomechanical structural adaptations processes in orthopedic surgery has becoming a major challenge since ages due to constraints such as limited supply, need for additional surgery and donor site morbidity. The complicated procedure also may causing undesirable drawbacks which includes disease transmission and risk of infection [61], [62].

Recently, biocomposite materials have been recognized as a promising alternative to inorganic and synthetic materials including fillers/reinforcements and matrix. Parallel with increasing in environmental awareness and expanding global waste problems, many industries are currently pursuing the practice of materials that will decrease the level of environmental contamination and economic cost [63]. A biocomposite is a material composed of two or more distinct constituent materials (one being naturally derived) which are combined to yield a new material with improved performance over individual constituent materials [64]. These kind of materials often mimic the structure of the living materials involved in the process keeping the strengthening properties of the matrix that was used, but always providing biocompatibility [65]. Researcher found that the ever-growing demand for low cost in materials processing put industry to have interest towards production and utilization of by-products as an alternative to new reinforcement/filler materials since they are readily available or naturally renewable at economical cost [66]. Eggshell waste reinforced composite materials consist of high strength and Modulus fixed in or bonded to a matrix with definite interfaces (boundaries) between them. In this form, eggshell powder (containing calcium carbonate) and matrix retain their physical and chemical identities and thus, they will produce a combination of properties that cannot be achieved with either of the constituents acting alone [67]. In general, eggshell fillers are the load-carrying members, while the surrounding matrix keeps them in the desired location and orientation, acts as a load transfer medium between them, and protects them from environmental damages due to elevated temperatures and humidity [68]. Thus, even though the fillers provide reinforcement for the matrix, the latter also serves a number of useful functions in eggshell reinforced composite material.

2.1 Eggshell Waste Reinforce in Metal Composite

There are extensive research has been done related to bone reconstruction process and its general issues in the interest of bone defects that caused by infections, tumor, trauma, surgery and so on. Throughout the ages, it is racing against times to develop the ideal orthopedic treatments with precise specifications such as being biocompatible, biomechanics of bone tissue, osteoconductive, osteoinductive and have minimal toxic reaction towards the host [69]. Metal matrix composites (MMCs) are known to be pioneer materials in rapidly replace monolithic materials due to their improved properties including specific strength, fatigue strength, wear resistance and creep resistance [70], [71]. The advantages in performance of metal matrix composites is their ability in tailored mechanical and physical properties. The ever-increasing demand for low cost reinforcement stimulated the interest towards production and utilization of using by-products from industry as reinforcement since they are readily available or are naturally renewable at affordable cost [72]. Metallic biomaterials are engineered systems designed to promote biomechanics study into practical based on the laws of mechanics and knowledge of anatomy and physiology of bone tissue [54]. However, the main disadvantage of metallic biomaterials are their lack of biological recognition on the material surface. To overcome this restraint, surface coating or surface modification presents a way to preserve the mechanical properties of established biocompatible metals improving the surface biocompatibility [54].

In addition to that, for the purpose of enhancing synergy between cells, facilitating their organization within the porous scaffold it is expected to integrate cell-recognizable ion/molecule attached to metal atom (ligands) and signaling growth factors on the surface of the scaffolds [73]. Inclusion of eggshell waste (EW) as source of calcium phosphate (CaP) allows a controlled and accelerated osseointegration between implant surface and living bone [74]. Although EW have shown excellent result to become source of CaP, it usage as filler in metal composite for bone tissue engineering application, it seems not being develop much. Only several research can be found have interest in combination of EW with metal for that application. Ferreira J. R. M. et al. 2017, use ostrich eggshell as calcium ion (Ca^{2+}) source in the preparation of zinc (Zn), CaP coatings on titanium (Ti) substrates through two step deposition method. Ostrich eggshell being treat to become CaP through precipitation method before being used as electrolyte with Zn to coat Ti. Based on the result, presence of Zn brushite (phase in metal) and Zn-hydroxyapatite (HA) as coating phases. It can be seen in Fig. 2 below how the microstructure of Zn brushite and Zn hydroxyapatite coating [75].

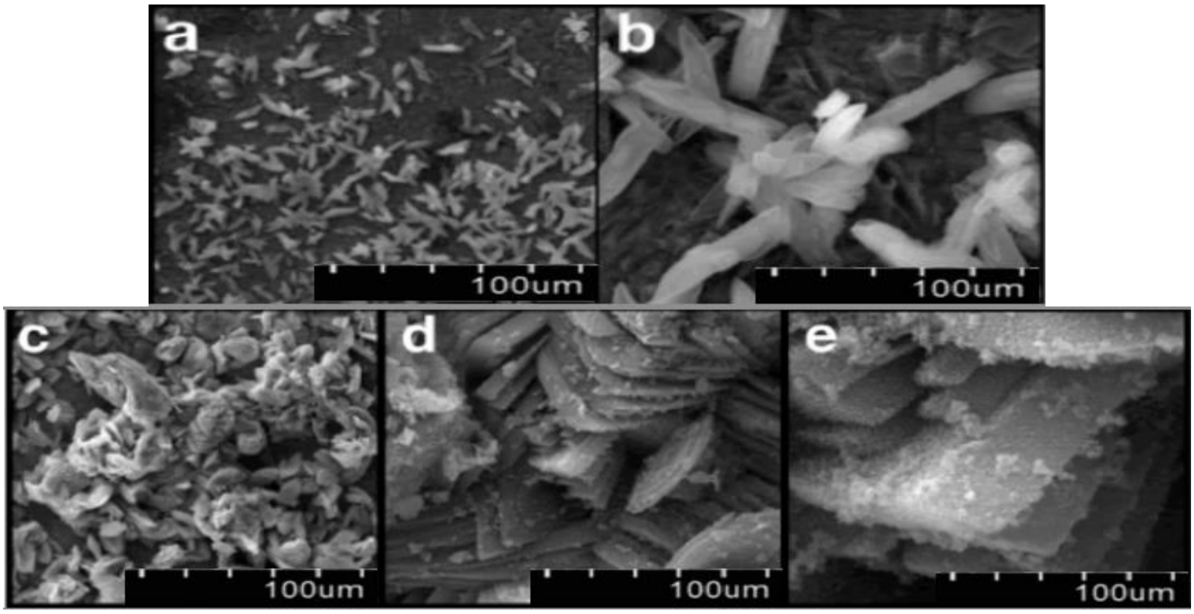

Fig. 2 - SEM images showing the deposition of (a, b) Zn-brushite coatings from precursor solution; (c,d,e) Zn hydroxyapatite coating [75]

Fig. 2 (a, b) showed platelet crystals on all samples according to characteristic of brushite crystals morphology. These results indicated a Zn influence on the precipitation of acidic calcium phosphate in this deposition method. In Fig. 2 (c,d,e) shows nanometric needle like morphology, characteristic of HA on the former brushite crystal surface. Zn-brushite phase is a metastable CaP and hence transforms into crystalline bone-like apatite. The incorporation of Zn ions into HA structure is known to induce distortions in its crystal lattice and lead to the Ca-deficient HA, increasing its solubility and bioactivity. Ostrich eggshells was shown to be effective as a Ca^{2+} ions source to obtain Zn containing HA coating on Ti substrates. Simulated body fluid (SBF) test for bioactivity result shown the satisfactory bioactivity while toxicity test shown non toxicity in this coating [75].

In 2017, other researcher also use ostrich eggshell to coat on Ti substrate. Marchal R. L. S. B. et al. 2017 use ostrich eggshell to form CaP through precipitation method as coater on Ti substrate. CaP become electrolyte in electrodeposition process of Ti. The result obtained the first step of deposition creates brushite and monetite of CaP phase and titanium hydride (TiH_2) due to interaction between substrate and X-ray during XRD process while the second step of deposition that creates HA as CaP major phase. It also reveals that Ti and titanium hydride (TiH_2) due to interaction between substrate and X-ray during XRD process [22]. In addition to that, it is expected that each step individually could create a stable calcium phosphate phase. The first step of coating creates brushite and monetite and the second step creates HA as it being dipped in alkaline solution to convert CaP from first step coating to HA in second step coating. This experiment shows that ostrich eggshell could be used as source of calcium ions. In bioactivity test, the result shows first step deposition phases showed the precipitation of bone-like apatite, due to their high dissolution coefficient while second step deposition process showed no bone-like apatite precipitation, but an initial stage of dissolution indicating lower bioactivity when compared to result of first stage.

2.2 Eggshell Waste Reinforce in Ceramic Composite

Porous structure of implant is one of the major aspect that need to be concerned before practice it for bone application. Porous structure is the key for cell adhesion, differentiation to bone tissue engineering, avoid stress shielding and improve mass transfer [76]. There is rising confirmation that some crucial aspects regarding the clinical success of bioceramics, such as the rate of resorption and the extent of angiogenesis. This success not only depend on the intrinsic properties of the material but also on the volume, size and shape of the pores it contains [77]. Fedorovich N. E. et al. 2018 and Kim J. A. et al. 2016 stated that porosity is important for cell and tissue conductive properties and promotes rapid biodegradation and scaffold bone regeneration [78], [79]. Ceramic known as one of the material that consist of open and closed pore in it microstructure [80]. Open pore is pore that can be seen while closed pore is pore inside the material that can’t be seen by bare eyes. For more specific, there are three types of pore based on its size; nanopores (pores < 0.1 µm), micropores (range 0.1 to 10 µm) and macropores (> 100 µm). For suitable application, modulation of the specific surface area and the porosity at a various size of pores has become a powerful tool that allows fine-tuning of the degradation of ceramic [81]. Different types of pores will give different result towards the implant; macroporous is ideal bone graft as it can promote tissue ingrowth while micro and nanopores with suitable controlling play compatible role in formation of bone and material resorption [82]. From the characteristic of ceramic that contain porosity that have similar properties with bone, several researcher have added eggshell waste in ceramic composite in order to research it contribution to that composite system.

Several researcher that have interest in combine EW with ceramic is Ayawanna J. et al. 2019. In that research, biomedical porous glass - ceramic orbital implants from egg shell-based calcium-silicate glasses being prepared. Calcium-bearing silicate glass material commercially made up from silica (SiO_2), calcium carbonate ($CaCO_3$) and sodium carbonate ($NaCO_3$). However, this research use EW that had being calcined at temperature 1000 °C to become substitution to commercial CaCO3 in calcium-bearing silicate glass preparation. Based on the crystallinity result, implants obtained a pure biocompatible $CaSiO_3$ phase at temperature of heating 1100 °C after add in sponge casting while morphological analysis shows this implants contained an open-macropore network with porosity over 30% that allowing the fibro vascular tissue ingrowth as shown in Fig. 3 below.

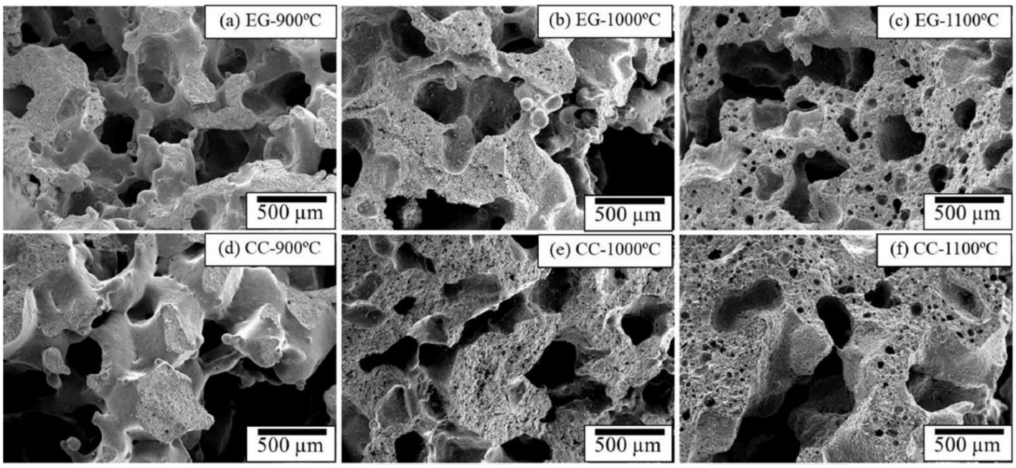

Fig. 3 - Morphology of (a to f) sintered porous glass - ceramic samples with high-magnification SEM images of surface in porous glass–ceramic samples; EG = eggshell, CC = $CaCO_3$ [83]

The transformation of porous structure in glass–ceramic implants from egg shells and $CaCO_3$ was relatively similar in the samples sintered between 900 and 1100 °C as can be seen in Fig. 3. When sintering temperature increase, the open pore network in samples decrease. The porosity of glass - ceramic samples sintered at 900 to 1100 °C reduced from 40% to 20%, which is less than that of bio-implants reported by past research. Besides, close pore accompanied with rough surface were developed in the glass - ceramic samples when the temperature increased from 900 to 1100 °C. This probably occur due to the pore residue from glass fusing with CaO to form wollastonite phase. At high temperature, it will cause high viscosity of glass melt so it will prevents smoother surface forming. Lastly, simulated body fluid result show good chemical stability for EW in calcium-bearing silicate glass implant. From this result, EW can be substitution of CaCO3 in calcium-bearing silicate glass [83].

In the same year, Naga S. M. et al. 2019 have use zirconia-toughened alumina (ZTA) with HA from extraction of eggshell waste. In this research, EW had being synthesize using sol gel technique and then being mixed with ZTA using ball milling. The result of phase composition and microstructure shows the formation of new phases, α-tricalcium phosphate (α-TCP), and hibonite ($CaAl_{12}O_{19}$), which developed as a result of the reaction between ZTA and HA. Fig. 4 below shows XRD result pattern of ZTA with different percentage.

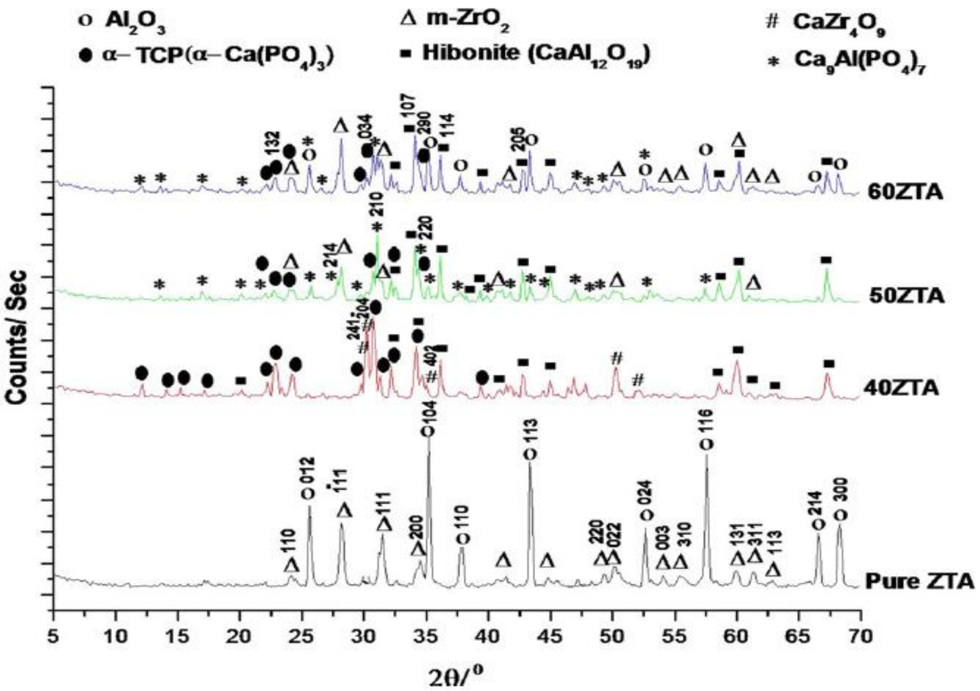

Fig. 4 - XRD result of ZTA with different percentage [84]

Based on Fig. 4, it was observed that alumina (Al_2O_3) and m-zirconia (m-ZrO_2) phases were the only components of the pure ZTA composite fired at 1650 °C. In contrast, the addition of various amounts of ZTA (40, 50, and 60 wt %) to the HA composites led to the formation of α-TCP and hibonite phases. When firing at high temperatures, HA decomposes into TCP, and CaO is released. The released CaO reacts with Al_2O_3 to form the hibonite phase, $CaAl_{12}O_{19}$. $CaZr_4O_9$ was observed in the 40ZTA composite only. The 50ZTA and 60ZTA composites exhibited α-TCP, hibonite, Al_2O_3, ZrO_2, and calcium aluminum phosphate [$Ca_9Al\ (PO_4)^7$] phases. For mechanical properties, 50 wt% of ZTA give highest value of compressive strength while biocompatibility test shown excellent biocompatibility [84].

Onwubu S. C. et al. 2019, mixed titanium dioxide (TiO_2) with hen eggshell after being ball milling for dental application. The result shows irregular shaped particles (calcite shape of eggshell powder) coexisted with the spherical shaped particles (TiO_2) due to their mixing reaction in ball milling process. Cytotoxicity test shows no significant result in different concentration. In this research, acidic test being done as teeth will react with acidic food. The result shows this material can be used for dental application as it shown great result against the acidic environment [85]. Lastly, Kalaiselvi V. and Mathammal R. 2016 also combine TiO_2 with EW but using different fabrication method. In this research, XRD result confirmed the presence of pure and Ti doped HA nanoparticles based on it grain size, cell parameters, and unit cell volume while SEM result shows formation of conglomerated and cluster like structure in pure and doped samples. From this result, combination of TiO_2 with HAp from EW is success [86].

2.3 Eggshell Waste Reinforce in Polymer Composite

Scaffold materials can be biologic or synthetic, degradable or non-degradable, depending on the intended use. The properties of polymers depend on the composition, structure, and arrangement of their constituent macromolecules. Besides, it can be categorized into different types in terms of their structural, chemical, and biological characteristics. There are three main types of polymers widely used as biomaterials; natural polymers, synthetic biodegradable and synthetic non-biodegradable polymers [87]. As mentioned, polymers have been extensively used as biomaterials mainly for the fabrication of medical device and tissue-engineering scaffolds. In biomedical applications, the keys in selecting the material that served as biomaterials are based on their chemistry, molecular weight, solubility, shape and structure, membrane properties (hydrophilicity/hydrophobicity), lubricity, surface energy, water absorption, degradation and erosion mechanism [88], [89]. To be significant in the application of tissue engineering and organ substitutions, polymeric scaffolds offer definite merits of biocompatibility, versatility of chemistry and biological properties. In addition to that, these merits are drawing a great attention from both researchers and manufacturers in fabricating polymeric scaffolds due to their unique properties including ability to provide more controllability on physiochemical characteristics of scaffolds such as high porosity with smaller size, solubility, enzymatic reactions and allergic response [59], [90].

Naturally derived mineral filler such as $CaCO_3$ proved to improve strength and stiffness of polymer composite as fillers. The finest grades fillers is utilized primarily to modify and improve properties of composite while the lowest grades are used for the sake of reducing cost [91]. Utilization of eggshell as filler can replace common inorganic filler for the reason that high content of $CaCO_3$ approximately 93-95% and the remaining are organic matters. In recent years, great deal of effort have been performed for transforming the eggshell wastes to a valuable product majoring in bones tissue regeneration as starting materials for calcium phosphate in polymeric scaffolds [5], [20], [92]. The vast application of eggshell as fillers in polymeric scaffolds is observable in both biodegradable polyester and thermoplastics. Biodegradable polyesters are reviewed to be the most commercially competitive polymers in bone scaffolds applications due to reproducibility in a cost-effective manner with extensive range of characteristics [93]. Several works have been reported on inclusion of eggshell waste in polycaprolactone (PCL) in which to encourage towards sustainable development. PCL is one of the most appealing biodegradable polyesters that has been reported as an excellent material to be used in bone regeneration owing to its excellent mechanical properties, nontoxicity, biodegradability and biocompatibility with human biological systems [93], [94]. However, its properties can be improved with the combination of other material. Morphology studies regarding addition of eggshells powder in polyester blending were shown in Fig. 5 [95]. Biscaia et al. 2015 successfully produced using an addictive manufacturing technique where eggshell powder was used as a filler [48]. In relation to the manufacturing process, morphological observation found that the addition of eggshell in PCL matrix alters the flow behavior of the composite thus, resulting in reduction in pore size. In the same manner of scaffold materials, the incorporation of eggshell powder in PCL matrix resulting in scaffolds with interconnected channel networks and alongside controllable porosity [96].

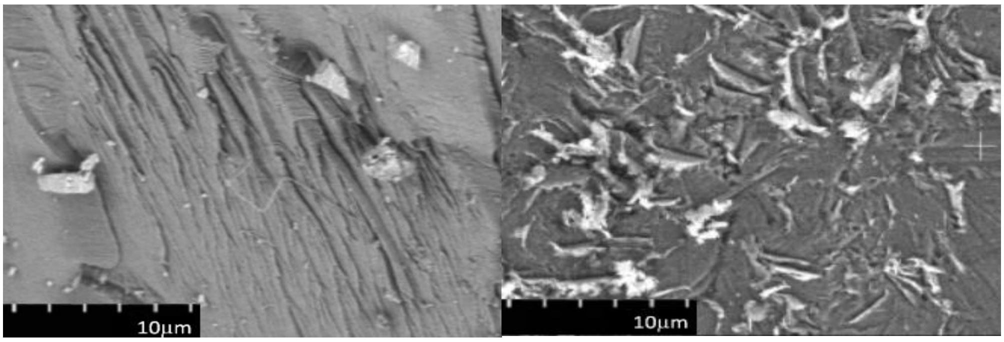

Fig. 5 - SEM microstructure of 20wt% of (left) uncarbonized eggshell; (right) carbonized eggshell addition polyester composite [95]

In other works by Koroleva et al. 2017 the PCL matrix were filled with doped $CaCO_3$. $CaCO_3$ from eggshell waste was doped by microelements (K^+, Mg^{2+}, Fe^{2+}, Zn^{2+}, Mn^{2+}, Li^+, Au^{3+}, SiO_2) to become nanocrystalline calcium carbonate-phosphate that aims to speed up the bone reconstruction process and bone tissue strengthening. It is essential to mimic the human bone biological environment so that it can have an active effect on the strengthening of bone tissue including activity through the skin. It also mentioned that, the morphology, stability, degree of crystalline along with mechanical properties of calcium hydroxyapatite require isomorphous substitution of calcium on such cations as Na^+, K^+, Mg^{2+}, Fe^{2+}, Zn^{2+} [97]. They have crucial function in the biological process of the osteogenesis [98]. Next, some other findings revealed that hydroxyapatite (HA) was successfully synthesized from eggshells with phosphoric acid by wet chemical precipitation method and the presence of HA was confirmed by FTIR spectroscopy and XRD [99]. The HA from eggshells (bio-HA) is appraised for good quality because of its resemblance in composition to the inorganic component of human hard tissues [20].

Trakoolwannachai V. et al, 2019 added that, the inclusion of bio-HA influenced degradation of PCL and led to the formation of interface between bio-HA filer and the PCL matrix [99]. Besides PCL, poly(lactic) acid also called PLA is another emerging biodegradable polyesters as potential tissue scaffolds [100]. PLA has higher transparency compare to other biodegradable polyesters along with its superior in weather resistance and workability. Morphological studied show that HA synthesized from eggshell waste incorporate in PLA matrix exhibit numerous pores and rough surface edges suitable for cell attachment [101]. The incorporation of eggshell waste as a filler also gaining interest in thermoplastics polymer as biomedical implant. In recent works conducted by Oladele et al. 2019 they investigates the eggshell as source of hydroxyapatite and high-density polyethylene (HDPE)/HA were developed [102]. The developed composite proved that the bio-HA particles enhance the mechanical properties of polymer composites to meet the structural challenges of bio-composites. In addition to that, Young's modulus and ultimate stress of filled HDPE increased parallel with increasing in eggshells content up to 40 wt%. Compatibilizer were added up to 2 wt% into the composite blends for the purpose of homogeneous distribution of the filler during mixing (Fig. 6) in manufacturing process [103]. Also, polypropylene (PP) thermoplastics polymer displayed similarity in addition of eggshells filler in developed composites.

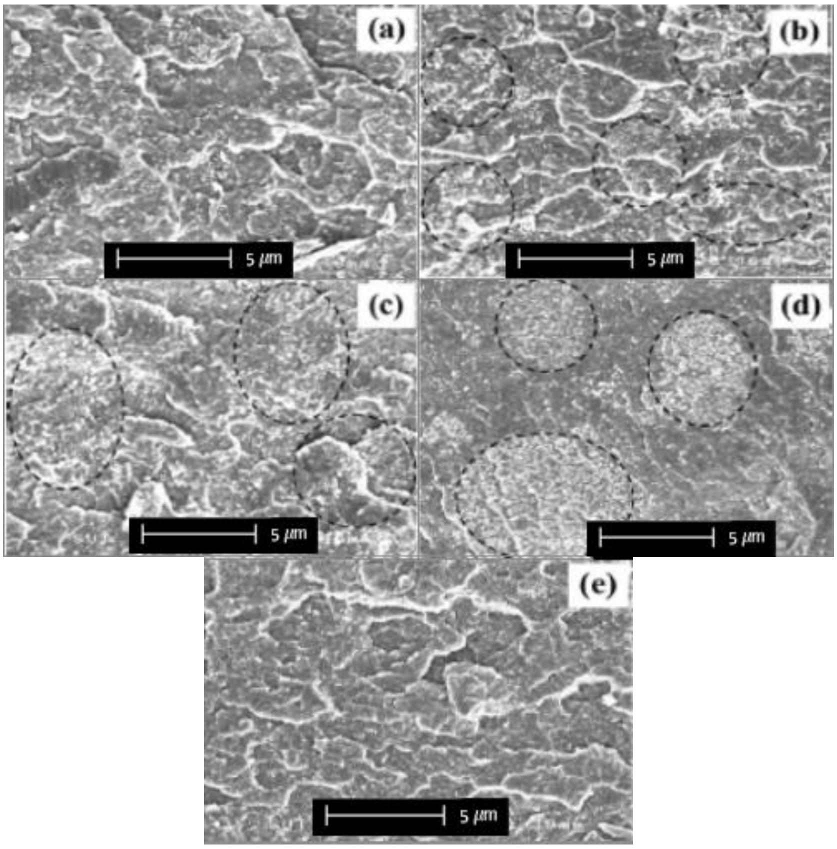

Fig. 6 - SEM micrographs at 300x magnification of HDPE filled with calcined eggshell powder at contents of (a) 10wt%; (b) 20wt%; (c) 30wt%; (d) 40wt%; (e) 40wt% with compatibilizer [103]

The addition of filler with size of less than 160 µm resulting in improvement in both tensile and flexural modulus in parallel with workability during extrusion process [104]. Besides, it was found that the particles size of calcium oxide (CaO) powders obtained from higher calcination temperature (starting at 800 °C) of eggshells compare to calcined at temperature below 800 °C (Fig. 7). These smaller particle size of less than 100 nm makes it possible in developing chitosan cryogel scaffolds reinforced with bio-HA for possible tissue engineering applications [46]. There are also studies reported on the fabrication of scaffolds containing bio-HA with some bioactive polymer or protein such as collagen, gelatin and chitosan. One particular work on carboxymethyl cellulose (CMC)/bio-HA scaffolds displayed degradable naturally overtime after performing degradability test in which necessary for tissue growth. Plus, the results from immersion in SBF (simulated body fluid) solution for 28 days indicate the high bioactivity of the scaffolds [45]. The broad range eggshells as reinforced also existed in production of hydrogels for bone tissue engineering. Eggshell microparticle reinforced gelatin-based hydrogels was successfully developed to obtain mechanically stable and biologically active 3D scaffolds that can differentiate pre-mature cells into osteoblasts [105].

3. Fabrication of Eggshell Waste Reinforcement for Biocomposite

3.1 Coating Process

Surface coating process is a process to protect surface of material from environmental attack especially metallic material from corrosion to decrease manufacturing cost [106]. There are various types of coating technique including physical/chemical vapor deposition, micro-arc oxidation, sol gel and electrodeposition processes [107]. Electrodeposition (Fig. 8) is one of protecting type using material ion to deposit on substrate and it worked when difference in potential between anode and cathode poles in this process induced ion transfer in unit cells [108]. Ferreira J. R. M. 2017, used this technique to coat titanium (Ti) substrate with zinc (Zn) and hydroxyapatite (HA) in order to increase bioactivity of the composite to use as implant [75]. Marchal R. L. S. B. 2017 also use same procedure to produce coating layer on Ti in precursor solution rich in Ca^{2+} and PO_4^{3-} ions with adding of ostrich eggshell and second deposition process to change calcium phosphate (CaP) to HA in potassium hydroxide solution. The advantage of this process is it increase adhesion between plate and the coating material while the drawbacks of this process will be costly and time consuming [22].

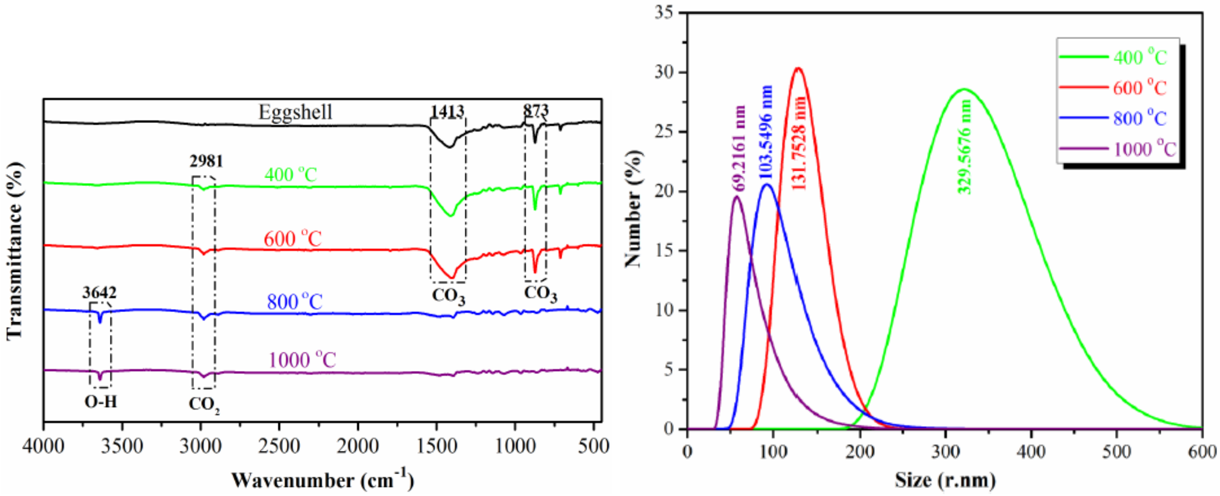

Fig. 7 - (left) FTIR analysis; (right) particle size distribution of CaO powders synthesized at 400 °C, 600 °C, 800 °C and 1000 °C [46]

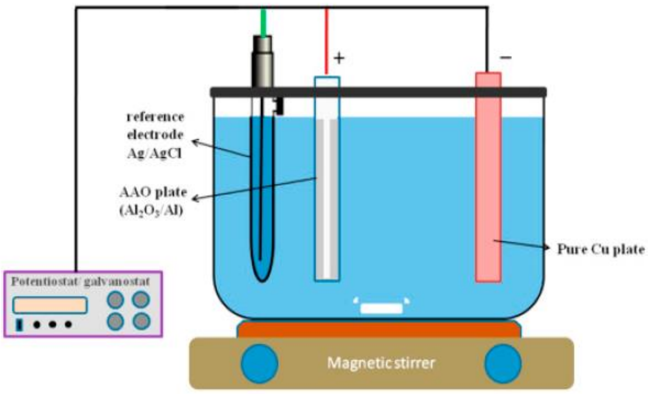

Fig. 8 - Schematic diagram of electrodeposition with example copper as cathode, aluminum oxide as anode and silver chloride as reference electrode [107]

3.2 Powder Processing

Powder processing (Fig. 9) is processing in producing material in powder form or decrease the size of powder (comminution process) either blending, mixing, grinding or other process [109]. In biocomposite fabrication, mostly mechanical processing of powder that being choose is grinding using ball mill and mixing using suitable speed with absent of ball. Ball milling is grinding method that grinds powder material into fine powders that mostly researcher used to produce fine size of powder, to make powder homogenous and mixing more than one type of powder [110]. Onwubu S. C. 2019 choose this process to mix eggshell powder with titanium dioxide for dental application. Eggshell waste being ball milled to fine sized before being mixed with titanium dioxide based on desired ratio in that research [85]. Naga S. M. 2019 also use this method to mix ZTA with HAp from EW using 300 rpm for 2 hours [84]. The benefit in utilising powder processing reveals that fine powder can be obtained however, contamination of product may occur due to wear and tear which occurs principally from the balls and partially from the casing during it process.

3.3 Melt Blending Process

The purpose of melt blending (or melt extruder) process is to prepared a homogeneous blend of polymer matrix and bioresorbable ceramic fillers that will exhibit the desired properties for specific tissue engineering applications. Undoubtedly, the ceramic and polymer blends may contribute to reinforced porous structures with improved bioactivity and controlled resorption rates [112]. Besides, this process results in composites with a higher modulus than solvent prepared compounds plus it is a solvent-free approach. In one of Ferri J. M. et al. 2016 work, variant compositions of PLA matrix and β-TCP bioceramic reinforcement were manufactured by means of melt extruder and later followed with injection molding. PCL/eggshell blends were also obtained using the same processing conditions (melt blending) before scaffolds were produced by means of a layer-by-layer manufacturing system resulting in 3D scaffold structure [48], [96], [113]. Sutapun W. et al. 2013 works with calcined eggshell waste melted with HDPE at contents of 10-40 wt%. The mixing process was performed at 170 °C under 70 rpm of rotor speed and 15 minutes of mixing time. Then, followed by injection molding process to prepared test specimen with melting temperature of 190 °C, a screw speed of 104 rpm, injection speed of 57 mm/s and holding pressure of 960 kg/cm^2 [103]. Another work conducted by mixing of polypropylene (PP) and eggshell, silane was used during mixing process as a coupling agent to bind with the polymer matrix. As a result, the composite material were stabilized [104]. Besides, Asha A. et al. 2014 also acknowledged the fabricating composite by injection molding as they succeed in producing polyamide/nylon matrix reinforced with eggshell composite for tissue engineering [58]. The main advantage of melt blending is compatible with current industrial processes that suitable to be process in most industry area. But having said that, this process needed to be closely control to avoid defect during production.

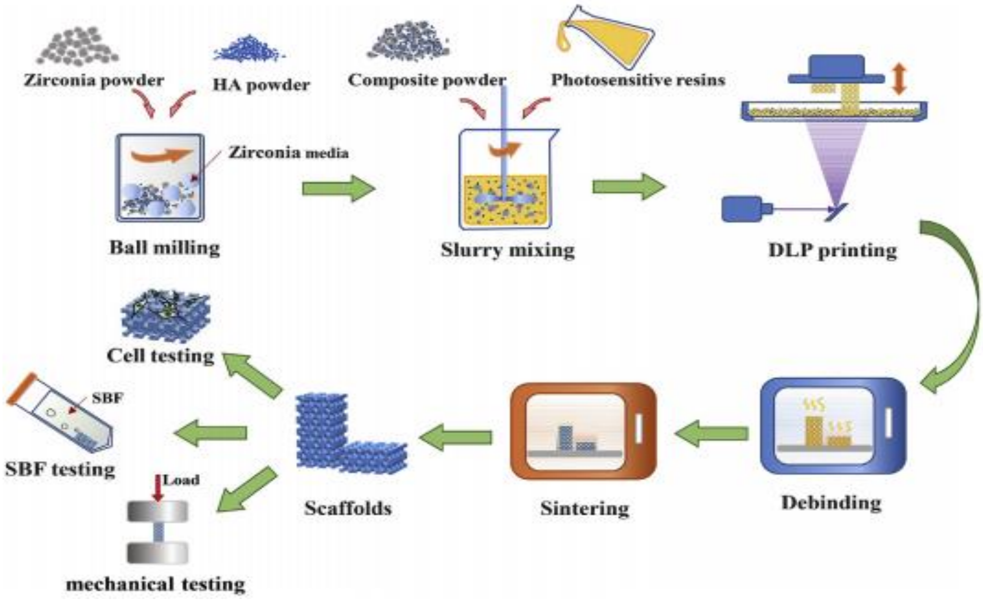

Fig.9 - Example illustration of the fabrication and testing process associated with powder processing [111]

3.4 Foam Replication Process

Foam replication (Fig. 10) is one of the various technique used to produce bone scaffold for porous bone substitution or bone grafting in the human body [114]. The advantage of this process is the shape of the product will follow exactly like it foam however, it is just costing as one foam just made for one product. From this method, scaffolds with similar to trabecular bone can be produced, as well as producing a controllable pore size and porosity scaffold [115]. Ayawanna J. et al. 2019 use this method to produce calcium-bearing silicate glass by dipping polyurethane (PU) in water-based glass slurry that contain polyvinyl alcohol (PVA) binder, water and glass [83]. After that it being dry for a day and being heated from temperature 900 to 1100 °C to remove PU foam and to form porous structure.

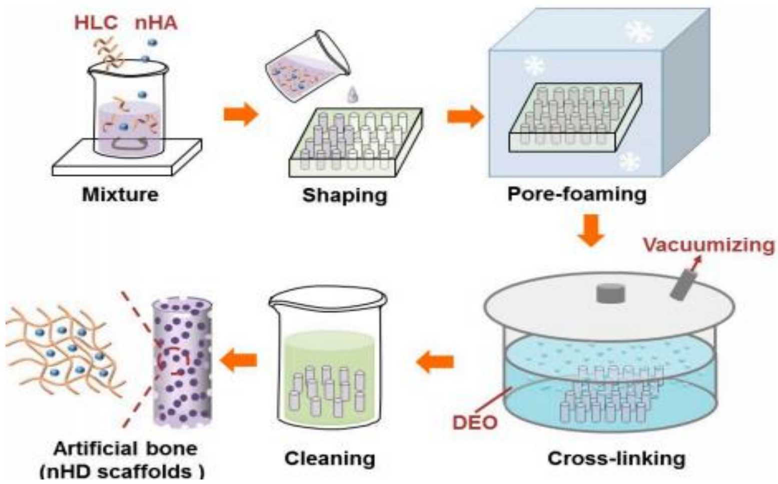

Fig. 10 - Schematic diagram of foam replication process in production of nano-hydroxyapatite (n-HA) with human-like collagen (HLC) scaffolds [116]

3.5 Wet Chemical Process

Wet chemical process is the process using liquid as medium for it processing. One of the wet chemical process that basically being used is precipitation method. Precipitation is the process of conversion of a solution into solid by converting the substance into insoluble form or by making the solution a super saturated and being precipitate [117]. Kalaiselvi V. and Mathammal R. 2016 use this method by mixing eggshell powder with titanium dioxide and distilled water. Then, ortho phosphoric acid being added while ammonia being added to adjust pH value. Lastly, the solution being filter and the precipitation product being calcined to get fine Ti doped HAp [86]. The advantage of this process is it well-established technology with ready availability of equipment and many chemicals that easily to process while in contrast high amount of waste is produced.

4. Conclusion

The output of this review can be comprehended by the favorable development of biomaterials for bone tissue replacements which increased and attracted a lot of interest due to the rise in the number of patients that requires bone replacements, especially in those suffering from bone cancer, trauma and ageing. Also, the rising advanced method that makes unparalleled scaffolding possible to be manufactured. To sum up everything that has been stated so far, the conversion of wastes and channeled towards the production into value added products of biomaterials served as implant component material. Eggshell is proved to be considered as an ideal source of calcite hence makes it an essential material for calcium phosphate powder mainly hydroxyapatite and β-tricalcium phosphate production. Despite that, present concern emphasizes the need in relatively simple method with less cost and possible to be recommended for the mass production of high yield percentage of crystalline calcium phosphates for future development. In addition to that, another challenges are to select the most convenient method in fabricate porous scaffolds with tailored scaffolds shape and controllable of its macro and microstructures thus, promoting cell proliferation upon implantation. With this development, discovering processing conditions that satisfy both the fabrication process and the final required scaffold properties is crucial.

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References

[1] Moh, Y. C. & Abd Manaf, L. (2014). Overview of household solid waste recycling policy status and challenges in Malaysia. Resources, Conservation and Recycling, 82, 20-61.
[2] Jaafar, I., Ibrahim, T. A., Ahmad, N. A., Kadir, A. A., & Tomari, M. R. M. (2018). Waste generation and characteristization: Case study of Seberang Takir, Kuala Nerus, Terengganu, Malaysia. International PostGraduate Conference on Applied Science & Physics , 1049, 1-7.
[3] Chu, M. M. (2019, July 30). Generating more waste than ever, The Star Online, 1, Retrieved from https://www.thestar.com.my/news/nation/2019/07/30/generating-more-waste-than-ever.
[4] King, K. S. & Liu, A. (2018, September 20). Global waste to grow by 70 percent by 2050 unless urgent action is taken. World Bank Report, Retrieved from https://www.worldbank.org/en/news/press-release/2018/09/20/global-waste-to-grow-by-70-percent-by-2050-un.
[5] Arabhosseini, A. & Faridi, H. (2018). Application of eggshell wastes as valuable and utilizable products: A review. Research in Agricultural Engineering, 64 (2), 104-114.
[6] Sethupathi, S., Kai, Y. C., Kong, L. L., Munusamy, Y., Bashir, M. J. K., & Iberahim, N. (2017). Preliminary study of sulfur dioxide removal using calcined egg shell. Malaysian Journal of Analytical Sciences, 21, 719-725.
[7] Mohammadpour, A. A., Zamanimoghadam, A., & Heidari, M. (2012). Comparative histomorphometrical study of genital tract in adult laying hen and duck. Veterinary Research Forum, 3, 27-30.
[8] Ketta, M. & Tumova, E. (2016). Eggshell structure, measurements, and quality-affecting factors in laying hens: a review. Czech Journal of Animal Science, 61, 299-309.
[9] Bashir, A. S. M. & Manusamy, Y. (2015). Characterization of raw egg shell powder (esp) as a good bio-filler. Journal of Engineering Research and Technology, 2, 56- 60.
[10] Bhagavatheswaran, E. S., Das, A., Hadi Rastin, H., Saeidi, H., Jafari, S. H., Vahabi, H, Najaf, F., Khonakdar, H. A., Formela, K., Jouyandeh, M., Zarrintaj, P., & Saeb, M. R. (2019). The taste of waste: the edge of eggshell over calcium carbonate in acrylonitrile butadiene rubber. Journal of Polymers and the Environment, 27, 2478-2489.
[11] Hincke, M.T., Nys, Y., Gautron, J., Mann, K., Rodriguez-Navarro, A., & McKee, M.D. (2012). The eggshell structure, composition and mineralization. Frontiers in Biosciences, 17, 1266-1280.

[12] Chien, Y. C., Hincke, M. T., & McKee, M. D. (2009). Ultrastructure of avian eggshell during resorption following egg fertilization. Journal of Structural Biology, 168, 525-538.

[13] Solomon, S. E. (2010). The eggshell: strength, structure and function. British Poultry Science, 51, 52-59.

[14] Shwetha, A., Dhananjaya, Shravana Kumara, S. M., & Ananda. (2018). Comparative study on calcium content in egg shells of different birds. International Journal of Zoology Studies, 3, 31-33.

[15] Kusuda, S., Iwasawa, A., Doi, O., Ohya, Y., & Yoshizaki, N. (2011). Diversity of the cuticle layer of avian eggshells. Journal of Poultry Science, 48, 119-124.

[16] Yang, T. Z., Chen, Y. H., Wiemann, J., Spiering, B., & Sander, P. M. (2018). Fossil eggshell cuticle elucidates dinosaur nesting ecology. Peer Journal, 6, 1-23.

[17] Hincke, M., Gautron, J., Rodriguez-Navarro, A. B., & McKee, M. D. (2011).The eggshell: structure and protective function. Improving the Safety and Quality of Eggs and Egg Products. United Kingdom: Woodhead Publishing Limited, 1, 151-182.

[18] Ketta, M. & Tůmová, E., (2018). Relationship between eggshell thickness and other eggshell measurements in eggs from litter and cages. Italian Journal of Animal Science, 17(1), 234-239.

[19] Lunam, C. A. & Ruiz, J. (2000). Ultrastructural analysis of the eggshell: Contribution of the individual calcified layers and the cuticle to hatchability and egg viability in broiler breeders. British Poultry Science, 41, 584-592.

[20] Abdulrahman, I., Tijani, H. I., Mohammed, B. A., Saidu, H., Yusuf, H., Jibrin, M. N. & Mohammed, S. (2014). From garbage to biomaterials: An overview on egg shell based hydroxyapatite. Journal of Materials, 1-7.

[21] Ajayan, N., Shahanamol, K. P, Arun, A. U., & Soman, S. (2020). Quantitative variation in calcium carbonate content in shell of different chicken and duck varieties. Advances in Zoology and Botany, 8, 1-5.

[22] Marchal, L. B. S. B., Ferreira, J. R. M., Louro, L. H. L., Costa, A. M., Rochal, D. N. D., Campos, J. B. D., Marcelo, F. & Silva, H. P. D. (2017). Apatite coatings from ostrich eggshell and its bioactivity assessment. Key Engineering Materials, 720, 185-188.

[23] Tangboriboon, N., Selarak, P., Shamunee, P., & Sirivat, A. (2016). Innovative green bio-filler and bio-flux calcium carbonate from eggshells to hard-soft porcelain preparation. Chemical Technology: An Indian Journal, 11, 109-119.

[24] Neunzehn, J., Szuwart, T., & Hans-Peter Wiesmann. (2015). Eggshells as natural calcium carbonate source in combination with hyaluronan as beneficial additives for bone graft materials, an in vitro study. Head & Face Medicine, 11, 1-10.

[25] Al-Obaidi, F. A., Basim, I. Mehdi, B. I., & Al- Shadeedi, S. M. (2012). Identification of inorganic elements in egg shell of some wild birds in Baghdad. Advances in Applied Science Research, 3, 1454 – 1458.

[26] El-Ishaq, A. & Kida, H.D. (2011). Comparative analysis of calcium carbonate content in eggshell of hen, duck and guinea fowl. Iraon, 3, 81-87.

[27] Negoita, I. S., Balaceanu, R. A., & Dojana, N. (2017). Calcium intake level as factor of influencing of the eggshell qualities in laying hens. Bulletin University of Agricultural Sciences and Veterinary Medicine Food Science and Technology, 74, 1-6.

[28] Hahn, E. N., Sherman, V. R., Pissarenko, A., Rohrbach, S. D., Fernandes, D. J., & Meyers, M. A. (2017). Nature's technical ceramic: the avian eggshell. Journal of the Royal Society Interface, 14, 1-11.

[29] Bharadwaj, A. V. S. L. S., Singh, M., Niju, S., Begum, K. M. M. S., & Anantharaman, N. (2019). Biodiesel production from rubber seed oil using calcium oxide derived from eggshell as catalyst – optimization and modeling studies . Green Processing and Synthesis, 8, 430-442.

[30] Peng, Y. P., Amesho, K., Chen, C. E., Jhang, S. R., Chou, F. C., & Lin, Y. C. (2018). Optimization of biodiesel production from waste cooking oil using waste eggshell as a base catalyst under a microwave heating system. Catalysts, 8, 81-96.

[31] Asri, N. P., Podjojono, B., Fujiani, R., & Nuraini. (2017). Utilization of eggshell waste as low-cost solid base catalyst for biodiesel production from used cooking oil. IOP Conferences Series: Earth and Environmental Science, 67, 1 – 8.

[32] Ngadi, N., Hamdan, N. F., Hassan, O., & Putra Jaya, R. (2016). Production of biodiesel from palm oil using egg shell waste as heterogeneous catalyst. Jurnal Teknologi, 78, 59-63.

[33] Tan, Y. H., Abdullah, M. O., & Nolasco-Hipolito, C. (2015). The potential of waste cooking oil-based biodiesel using heterogeneous catalyst derived from various calcined eggshells coupled with an emulsification technique: A review on the emission reduction and engine performance. Renewable and Sustainable Energy Reviews, 47, 589-603.

[34] Makuchowska-Fryc, J. (2019). Use of the eggshells in removing heavy metals from waste water - the process kinetics and efficiency. Ecological Chemistry and Engineering S, 26, 165-174.

[35] Tizo, M. S., Blanco, L. A. V., Cagas, A. C. Q., Dela Cruz, B. R. B., Encoy, J. C., Gunting, J. V., Arazo, R. O., & Mabayo, V. I. F. (2018). Efficiency of calcium carbonate from eggshells as an adsorbent for Cadmium removal in aqueous solution. Sustainable Environment Research, 10, 1-27.

[36] Borhade, A. V. & Kale, A. S. (2017). Calcined eggshell as a cost effective material for removal of dyes from aqueous solution. Applied Water Science, 7, 4255 – 4268.

[37] Ali, Z. T. A., Ibrahim, M. A., & Madhloom, H. M. (2016). Eggshell powder as an adsorbent for removal of Cu (II) and Cd (II) from aqueous solution: equilibrium, kinetic and thermodynamic studies. Al-Nahrain University, College of Engineering Journal (NUCEJ), 91, 186-193.
[38] Pettinato, M., Chakraborty, S., Arafat, H. A., & Calabro. V. (2015). Eggshell: A green adsorbent for heavy metal removal in an MBR system. Ecotoxicology and Environmental Safety, 121, 57-62.
[39] Andhare, A. A., Shinde, R. S., & Deshmukh, A. J. (2019). Banana peel, duckweed and egg shell: cheap sources of organic fertilizer. International Journal of Green and Herbal Chemistry, 8, 55-60.
[40] Khairnar, M. D. & Nair, S. S. (2019). Study on eggshell and fruit peels as a fertilizer. Proceedings of International Conference on Sustainable Development, 17, 25-27.
[41] Radha, T. & Karthikeyan, G. (2019). Hen eggshell waste as fertilizer for the growth of phaseolus vulgaris (cow pea seeds). Research Journal of Life Science, Bioinformatics, Pharmaceutical and Chemical Science, 5, 398-406.
[42] Wijaya, V. T. & Teo, S. S. (2019). Evaluation of Eggshell as Organic Fertilizer on Sweet Basil. International Journal of Sustainable Agricultural Research, 6, 79-86.
[43] Gaonkar, M. & Chakraborty, A. P. (2016). Application of eggshell as fertilizer and calcium supplement tablet. International Journal of Innovative Research in Science, Engineering and Technology, 5, 3520-32525.
[44] Horta, M., Aguilar, M., Mouraa, F., Camposc, J., Ramosc, V., & Quizundac, A. (2019). Synthesis and Characterization of Green Nanohydroxyapatite from Hen Eggshell by Precipitation Method. Materials Today: Proceedings, 14, 716-721.
[45] Sayed, M., El-Maghraby, H. F., Bondioli, F. & Naga, S. M. (2018). 3D Carboxymethyl cellulose/hydroxyapatite (CMC/HA) scaffold composites based on recycled eggshell. Journal of Applied Pharmaceutical Science, 8(3), 23-30.
[46] Demir, D., Ceylan, S., Ofkeli, F., Sen, D. & Karagulle, N. B. (2017). Eggshell derived nanohydroxyapatite reinforced chitosan cryogel biocomposites for tissue engineering applications. Journal of the Turkish Chemical Society Chemical Engineering B, 1, 77-88.
[47] Ferreira, J.R.M., Louro, L.H.L., Costa, A.M., de Campos, J.B. & Silva, M.H., 2016. Ostrich eggshell as calcium source for the synthesis of hydroxyapatite and hydroxyapatite partially substituted with zinc. Cerâmica, 62(364), 386-391.
[48] Biscaia, S. I., Viana, T. F., Almeida, H. A., & Bartolo, P. J. (2015). Production and characterisation of PCL/ES scaffolds for bone tissue engineering. Materials Today: Proceedings 2, 2018-2216.
[49] Amini, A. R., Laurencin, C.T. & Nukavarapu, S. P. (2012). Bone Tissue Engineering: Recent Advances and Challenges. Critical Review Biomedical Engineering, 40, 363-408.
[50] Chahal, S., Kumar, A., & Hussian, F. S. J. (2019). Development of biomimetic electrospun polymeric biomaterials for bone tissue engineering. A review. Journal of Biomaterials Science. Polymer Edition, 30, 1308-1355.
[51] Bullock, C. J. & Bussy, C. (2019). Biocompatibility considerations in the design of graphene biomedical materials. Advance Materials Interfaces, 6, 1-15.
[52] Bergmann, C.P., & Stumpf, A. (2013). Biomaterials. In: Dental Ceramics. Topics in Mining, Metallurgy and Materials Engineering. Berlin, Heidelberg: Springer.
[53] Pandey, E., Srivastava, K., Gupta, S., Srivastava, S. & Mishra, N. (2016). Some biocompatible materials used in medical practices - A review. International Journal of Pharmaceutical Sciences and Research. 3, 2748-2755.
[54] Prasad, K., Bazaka, O., Chua, M., Rochford, M., Fedrick, L., Spoor, J., Symes, R., Tieppo, M., Collins, C., Cao, A., Markwell, D., Ostrikov, K. & Bazaka, K. (2017). Metallic biomaterials: current challenges and opportunities. Materials, 10, 1-33.
[55] Roy, M., Bandyopadhyay, A. & Bose, S. (2017). Materials for Bone Disorders (pp. 265-314). Berlin, Heidelberg: Springer.
[56] Mohiuddin, M., Kumar, B., & Haque, S. (2017). Biopolymer Composites in Electronics (pp. 459-486). Berlin, Heidelberg: Springer.
[57] Basha, R. Y., Sampath Kumar, T. S. & Doble, M. (2015). Design of biocomposite materials for bone tissue regeneration. Materials Science and Engineering C, 57, 452-463.
[58] Asha, A. & Chandra Sekar, V. (2014). Investigation on the mechanical properties of egg shell powder reinfroced polymeric composites. International Journal of Engineering Research & Technology (IJERT), 288-291.
[59] Ghassemi, T., Shahroodi, A., Ebrahimzadeh, M. H., Mousavian, A., Movaffagh, J. & Moradi, A. (2018). Current concept in scaffolding for bone tissue engineering. The Archives of Bone and Joint Surgery, 6(2), 90-99.
[60] Winkler, T., Sass, F.A., Duda, G.N. & Schmidt-Bleek, K., 2018. A review of biomaterials in bone defect healing, remaining shortcomings and future opportunities for bone tissue engineering: The unsolved challenge. Bone & joint research, 7(3), 232-243.
[61] Karpinski, R., Jaworski, L. & Czubacka, P. (2017). The structural and mechanical properties of the bone. Journal of Technology and Exploitation, 3(1), 43-50.

[62] Dimitriou, R., Mataliotakis, G. I., Angoules, A. G., Kanakaris, N. K., & Giannoudis, P. V. (2011). Complications following autologous bone graft harvesting from the iliac crest. Injury: International Journal of the Care of the Injured, 42, 3-15.
[63] Reddy, T. R. K., Kim, H. J. & Park, J. W. (2016). Renewable biocomposite properties and their applications. Intech Open, 177-197.
[64] Rudin, A. & Choi, P., (2012). The elements of polymer science and engineering. Academic press.
[65] Bharath, K. N. & Basavarajappa, S. (2016). Applications of biocomposite materials based on. Science and Engineering of Composite Materials, 1-11.
[66] Dwivedi, S. P., Sharma, S. & Mishra, R. K. (2016). A comparative study of waste eggshells, CaCO3, and SiC-reinforced AA2014 green metal matrix composites. Journal of Composite Materials, 1-15.
[67] Hameed, H. K. & Jassim, W. H. (2019). Improvement of some mechanical properties of epoxy using uncarbonized and carbonized eggshell powder. AIP Conference Proceedings 2190, 1-9.
[68] Senthil, J. & Madan Raj, P. (2015). Preparation and characterization of reinforced egg shell polymer composites. International Journal on Mechanical Engineering and Robotics (IJMER), 3(3), 7-17.
[69] Grado, G. F., Keller, L., Idoux-Gillet, Y., Wagner, Q., Musset, A. M., Benkirane-Jessel, N., Bornert, F. & Offner, D. (2018). Bone substitutes: a review of their characteristics, clinical use, and perspectives for large bone defects management. Journal of Tissue Engineering, 9, 1-18.
[70] Zhang, M. Y., Ye C., Erasquin, U. J., Huynh, T., Cai, C. & Cheng, G. J. (2011). Laser engineered multilayer coating of biphasic calcium phosphate/titanium nanocomposite on metal substrates. Applied Materials & Interfaces, 3, 339-350.
[71] Srivastava, A. (2017). Recent advances in metal matrix composites (MMCs): a review. Biomedical Journal of Scientific & Technical Research, 1(2), 520-522.
[72] Linder, L. K. B., Birgersson, U., Lundgren, K., Illies, C. & Engstrand, T. (2018). Patient-specific titanium-reinforced calcium phosphate implant for the repair and healing of complex cranial defects. Bone Regenerative Implant for Cranial Defects, 1-9.
[73] Tamaddon, M., Samizadeh, S., Wang, L., Blunn, G. & Liu, C. (2017). Intrinsic osteoinductivity of porous titanium scaffold for bone tissue engineering. International Journal of Biomaterials, 1-12.
[74] Ayu, H. M., Izman, S., Daud, R., Krishnamurithy, G., Shah, A., Tomadi, S. H., & Salwania M. S. (2017). Surface modification on CoCrMo alloy to improve the adhesion strength of hydroxyapatite coating. Procedia Engineering, 184, 399 – 408.
[75] Ferreira, J. R. M., Louro, L. H. L., Costa, A. M., Marçal, R. L. S. B., Rocha, D. N. D., Barbosa, R. M., Campos, J. B., & Silva, P. D. M. H. (2017). Zinc-doped calcium phosphate coating on titanium surface using ostrich eggshell as a Ca2+ ions source. Biomaterials and Medical Application, 1, 1-12.
[76] Li, J., Chen, D., Luan, H., Zhang, Y., & Fan, Y. (2018). Numerical evaluation and prediction of porous implant design and flow performance. BioMed Research International, 2018, 1-13.
[77] Zhang, J., Luo, X., Barbieri, D., Barradas, A. M. C., de Bruijn, J. D., van Blitterswijk, C. A., & Yuan, H. (2014). The size of surface microstructures as an osteogenic factor in calcium phosphate ceramics. Acta Biomaterialia, 10(7), 1-10.
[78] Fedorovich, N. E., Schuurman, W., Wijnberg, H. M., Prins, H. J., van Weeren, P. R., Malda, J., Alblas, J. & Dhert, W. J. (2012). Biofabrication of osteochondral tissue equivalents by printing topologically defined, cell-laden hydrogel scaffolds. Tissue Engineering - Part C: Methods, 18(1), 33-44.
[79] Kim, J. A., Lim, J., Naren, R., Yun, H. S., & Park, E. K. (2016). Effect of the biodegradation rate controlled by pore structures in magnesium phosphate ceramic scaffolds on bone tissue regeneration in vivo. Acta Biomaterialia, 44, 155-167.
[80] Vakifahmetoglu, C., Semerci, T., & Soraru, G. D. (2020). Closed porosity ceramics and glasses. Journal of the American Ceramic Society, 103(5), 2941-2969.
[81] Diez-Escudero, A., Espanol, M., Beats, S., & Ginebra, M. P. (2017). In vitro degradation of calcium phosphates: Effect of multiscale porosity, textural properties and composition. Acta Biomaterialia, 60, 81-92.
[82] Ginebra, M. P., Espanol, M., Maazouz, Y., Bergez, V., & Pastorino, D. (2018). Bioceramics and bone healing. EFORT open review, 3(5), 173-183.
[83] Ayawanna, J., Kingnoi, N., & Laorodphan, N. (2019). A feasibility study of egg shell-derived porous glass–ceramic orbital implants. Materials Letters, 241, 39-42.
[84] Naga, S. M., Sayed, M., & El-Maghraby, H. F. (2019). Physicomechanical, phase composition, and in vitro study of high-temperature sintered ZTA/HA composites. Metallurgical and Materials Transactions A, vol. 50(11), 5533-5541.
[85] Onwubu, S. C., Mdluli, P. S., Singh, S., & Tlapana, T. (2019). A novel application of nano eggshell/titanium dioxide composite on occluding dentine tubules: An in vitro study. Brazilian Oral Research, 33, 1-10.
[86] Kalaiselvi, V. & Mathammal, R. (2016). Effect of titanium dioxide doping on nano hydroxyapatite synthesised from egg shells via wet chemical method. International Journal & Magazine of Engineering, Technology, Management and Research, 3(8), 133-138.

[87] Stratton, S., Shelke, N. B., Hoshino, K., Rudraiah, S. & Kumbar, S. G. (2016). Bioactive polymeric scaffolds for tissue engineering. Bioactive Materials, 1, 93-108.
[88] Mota, R. C. A. G., Silva, E. O. & Menezes, L. R. (2018). Polymer nanocomposites used as Scaffolds for bone tissue regeneration. Materials Sciences and Applications, 9, 679-697.
[89] Bose, S., Roy, M. & Bandyopadhyay, A. (2012). Recent advances in bone tissue engineering scaffolds. Trends in Biotechnology, 30(10), 546-554.
[90] Dhandayuthapani, B., Yoshida, Y., Maekawa, T. & Kumar, D. S. (2011). Polymeric scaffolds in tissue engineering application: a review. International Journal of Polymer Science, 1-20.
[91] Norihan, Y., Syuhada, M. T. & Nor Habibah, M. R. (2019). Green eggshell/polypropylene biocomposite. International Journal of Recent Technology and Engineering (IJRTE), 8(15), 386-389.
[92] Bardhan, R., Mahata, S. & Monda,l B. (2011). Processing of natural resourced hydroxyapatite from eggshell waste by wet precipitation method. Advances in Applied Ceramics, 110(2), 80-87.
[93] Manavitehrani, I., Fathi, A., Badr, H., Daly, S., Shirazi, A. N. & Dehghani, F. (2016). Biomedical applications of biodegradable polyesters. Polymers, 8(20) 1-32.
[94] Rydz, J., Sikorska, W., Kyulavska, M. & Christova, D. (2015). Polyester-based (bio)degradable polymers as environmentally friendly materials for sustainable development. International Journal of Molecular Sciences, 16, 564-596.
[95] Hassan, S. B., Aigbodion, V. S. & Patrick, S. N. (2012). Development of polyester/eggshell particulate composites. Tribology in Industry, 34(4), 217-225.
[96] Viana, T., Biscaia, S., Almeida, H. A. & Bartolo, P. J. (2014). PCL/eggshell scaffolds for bone regeneration. Proceedings of the ASME 2014 12th Biennial Conference on Engineering Systems Design and Analysis, 1-6.
[97] Koroleva, L. F., Dobrinskaya, M. N. & Kamantsev, I. S. (2017). Doped calcium carbonate-phosphate used for bone tissue technology. Integrative Clinical Medicine, 1(2), 1-7.
[98] Shepherd, J. H., Shepherd, D. V. & Best, S. M. (2012). Substituted hydroxyapatites for bone repair. Journal of Materials Science: Materials in Medicine, 23, 2335–2347.
[99] Trakoolwannachai, V., Kheolamai, P. & Ummartyotin, S. (2019). Characterization of hydroxyapatite from eggshell waste and polycaprolactone (PCL) composite for scaffold material. Composites Part B, 173, 1-7.
[100] Mohan, S., Oluwafemi, O. S., Kalarikkal, N., Thomas, S. & Songca, S. P. (2016). Biopolymers-Application in nanoscience and nanotechnology. INTECH Open Science, 47-72.
[101] Apalangya, V. A., Rangarl, V. K., Tilmob, B. J., Jeelani, S. & Samuel, T. (2019). Eggshell based nano-engineered hydroxyapatite and poly(lactic) acid electrospun fibers as potential tissue scaffold. International Journal of Biomaterials, 1-12.
[102] Oladele, I. O., Agbabiaka, O. G., Adediran, A. A., Akinwekomi, A. D. & Balogun, A. O. (2019). Structural performance of poultry eggshell derived hydroxyapatite based high density polyethylene bio-composites. Heliyon, 5, 1-7.
[103] Sutapun, W., Pakdeechote, P., Suppakarn, H. & Ruksakulpiwat, Y. (2013). Application of calcined eggshell powder as functional filler for high density polyethylene. Polymer-Plastics Technology and Engineering, 52(10), 1025-1033.
[104] Sivarao, Mohd Rizal, S., Kamely, A., Tajul, A. & Taufik. (2011). Characterizing chicken eggshell reinforced polypropylene (PP). Advanced Materials Research, 264-265, 871-879.
[105] Wu, X., Stroll, S., Lantigua, D., Suvarnapathaki, S. & Camci-UnalG. (2019). Eggshell particle-reinforced hydrogels for bone tissue engineering: an orthogonal approach. Biomaterials Science, 1-11.
[106] Qian, Y., Li, Y., Jungwirth, S., Seely, N., Fang, Y., & Shi, X. (2015). The application of anti-corrosion coating for preserving the value of equipment asset in chloride-laden environments: A review. International Journal of Electrochemical Science, 10, 10756-10780.
[107] Fotovvati, B., Namdari, N., & Dehghanghadikolaei, A. (2019). On coating techniques for surface protection: A review. Journal of Manufacturing and Materials Processing, 3, 1-22.
[108] Radwan, A.B., Ali, K., Shakoor, R., Mohammed, H., Alsalama, T., Kahraman, R., Yusuf, M.M., Abdullah, A.M., Montemor, M.F., & Helal, M. (2018). Properties enhancement of Ni-P electrodeposited coatings by the incorporation of nanoscale Y_2O_3 particles. Applied Surface Science, 457, 956-967.
[109] Kanda, Y. & Kotake, N. (2007). Handbook of powder technology (pp 529-550). Amsterdam, Netherland: Elsevier Limited Company
[110] Gou, J., Zhuge, J., & Liang, F. (2012). Processing of polymer nanocomposites (pp 95-119). Cambridge, United Kingdom: Woodhead Publishing.
[111] Cao, Y., Shi, T., Jiao, C., Liang, H., Chen, R., Tian, Z., Zou, A., Yang, Y., Wei, Z., Wang, C. & Shen, L. (2019). Fabrication and properties of zirconia/hydroxyapatite composite scaffold based on digital light processing. Ceramic International, 1-9.
[112] Mathieu, L. M., Bourban, P. E. & Manson, J. A. E. (2006). Processing of homogeneous ceramic/polymer blends for bioresorbable composites. Composites Science and Technology, 66, 1606-1614.

[113] Ferri, J. M., Gisbert, I., Garcia-Sanoguera, D., Reig, M. J. & Balart, R. (2016). The effect of beta-tricalcium phosphate on mechanical and thermal performances pf poly(lactic acid). Journal of Composite Materials, 1-10.
[114] Sharmiwati, M. S., Mizan, R. M., & Noorhelinahani, A. B. (2014). Preparation and characterization of ceramic sponge for water filter. International Journal of Scientific & Technology Research, 3(6), 103-106.
[115] Wattanutchariya, W. (2013). Relationship between polymeric foam characteristics and properties of porous bone substitute fabricated by polymeric foam replication. 2013 IEEE International Conference on Industrial Engineering and Engineering Management, 2013, 1072-1076.
[116] Liu, Y., Gu, J. & Fan, D. (2020). Fabrication of high-strength and porous hybrid scaffolds based on nano-hydroxyapatite and human-like collagen for bone tissue regeneration. Polymers, 12(61), 1-18.
[117] Jarvenin, G. (2008). Precipitation and crystallization processes. Consortium for risk evaluation with stakeholder participation (CRESP), 16, 1-14.