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Preparation and characterization of cockle shell aragonite nanocomposite porous 3D scaffolds for bone repair

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ABSTRACT

The demands for applicable tissue-engineered scaffolds that can be used to repair load-bearing segmental bone defects (SBDs) is vital and in increasing demand. In this study, seven different combinations of 3 dimensional (3D) novel nanocomposite porous structured scaffolds were fabricated to rebuild SBDs using an extraordinary blend of cockle shells ($CaCo_3$) nanoparticles (CCN), gelatin, dextran and dextrin to structure an ideal bone scaffold with adequate degradation rate using the Freeze Drying Method (FDM) and labeled as 5211, 5400, 6211, 6300, 7101, 7200 and 8100. The micron sized cockle shells powder obtained (75 μm) was made into nanoparticles using mechano-chemical, top-down method of nanoparticles synthesis with the presence of the surfactant BS-12 (dodecyl dimethyl bataine). The phase purity and crystallographic structures, the chemical functionality and the thermal characterization of the scaffolds' powder were recognized using X-Ray Diffractometer (XRD), Fourier transform infrared (FTIR) spectrophotometer and Differential Scanning Calorimetry (DSC) respectively. Characterizations of the scaffolds were assessed by Scanning Electron Microscopy (SEM), Degradation Manner, Water Absorption Test, Swelling Test, Mechanical Test and Porosity Test. Top-down method produced cockle shell nanoparticles having averagely range 37.8 ± 3–55.2 ± 9 nm in size, which were determined using Transmission Electron Microscope (TEM). A mainly aragonite form of calcium carbonate was identified in both XRD and FTIR for all scaffolds, while the melting (Tm) and transition (Tg) temperatures were identified using DSC with the range of Tm 62.4–75.5 °C and of Tg 230.6–232.5 °C. The newly prepared scaffolds were with the following characteristics: (i) good biocompatibility and biodegradability, (ii) appropriate surface chemistry and (iii) highly porous, with interconnected pore network. Engineering analyses showed that scaffold 5211 possessed 3D interconnected homogenous porous structure with a porosity of about 49%, pore sizes ranging from 8.97 to 337 μm, mechanical strength 20.3 MPa, Young's Modulus 271 ± 63 MPa and enzymatic degradation rate 22.7 within 14 days.

Abbreviations: SBD, Segmental Bone Defects; 3D, 3 Dimensional; $CaCO_3$, Calcium carbonate; CCN, Calcium Carbonate Nanoparticles; FDM, Freeze Drying Method; 5211, cockle shells nanoparticles 50%, gelatin 25%, dextran 10%, and dextrin 15%; 5400, cockle shells nanoparticles 50%, gelatin 40%, dextran 5%, and dextrin 5%.; 6211, cockle shells nanoparticles 60%, gelatin 20%, dextran 10%, and dextrin 10%; 6300, cockle shells nanoparticles 60%, gelatin 30%, dextran 5%, and dextrin 5%; 7101, cockle shells nanoparticles 70%, gelatin 15%, dextran 5%, and dextrin 10%; 7200, cockle shells nanoparticles 70%, gelatin 20%, dextran 5%, and dextrin 5%; 8100, cockle shells nanoparticles 80%, gelatin 10%, dextran 5%, and dextrin 5%; μm, Micrometer; BS-12, dodecyl dimethyl bataine; XRD, X-Ray Diffraction; FTIR, Fourier Transform Infrared; DSC, Differential Scanning Calorimetry; SEM, Scanning Electron Microscopy; nm, Nanometer; TEM, Transmission Electron Microscopy; T_m, Melting Temperature; °C, Degree Celsius; T_g, Glass transition Temperature; %, Percentage; MPa, Megapascals (MPa or N/mm^2) pascal (Pa) unit = one Newton per square meter; ECM, Extracellular Matrix; HA, Hydroxyapatite; $Ca_{10}PO_{46}OH_2$, Chemical structure of Hydroxyapatite; NC, Natural coral; mL, Milliliter; DW, Deionized Water; min, Minutes; cm, Centimeter; W_d, Dry Weight; R, Radius; T, Thickness; W_w, Wet Weight; P_{et}, Density of Ethanol; PBS, Phosphate Buffer Solution; W_1, Dry Weight; W_2, Wet Weight; U/mL, Unit per milliliter; W_0, Dry Weight (Initial Weight); ANOVA, One-Way Analysis of Variance; ACN, Aragonite Calcium Carbonate Nanoparticles; S.E., Standard Error; C-H, Carbon-Hydrogen group; C-O, Carbon-Oxygen group; JCPDS, Joint Committee of Powder Diffraction Society; H_f, Heat of fusion

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1. Introduction

Recently, biomedical approaches that have involved in repairing and restoring the functions of damaged tissues are categorized under tissue engineering field. Of all bone, tissue engineering is one of the promising fields that aim to create biological replacements that have the ability to restore, repair, maintain or improve tissue functions. The steps often start with manipulating and manufacturing of an appropriate three dimensional (3D) porous scaffold suitable for bone tissue regeneration. However, scaffold modifications may be the best way to accelerate degradation, for instances, adding ceramic particles to increase the surface area that is available for hydrolysis to enhance functional repair of segmental defects [1,2]. There are many types of biomaterials that present endless opportunities for innovations of novel matrix or a substrate for cell seeding [3,4]. These biomaterials serve as extracellular matrix (ECM) capable of supporting the new bone morphogenesis [5,6]. Bone is a self-repairing organ that has the ability to adapt its mass, form and properties in response to changes, such as mechanical necessities, and endures specific physical action in life without breaking or causing pain [7]. These bio-capabilities of the bone come from the fact that bone is a living organ and contain cells that activate renewal and repair capabilities. It is made up of organic and inorganic (mineral) materials. The organic substance is concentrated in the bone matrix that consists mostly of 90% collagen fibers and other non-collagenous proteins. The inorganic substance of the bone is a calcium phosphate so called hydroxyapatite (HA), Ca_{10} $(PO_4)_6(OH)_2$. The HA minerals is considered to fill the places between the collagen fibrils. The mechanical properties of any bone come from the impregnation process of the soft organic substances with HA minerals which are firm and brittle [8].

A scaffold is basically an extracellular matrix that offers a three dimensional structure capable of performing significant function. This practicability is due to a near-net-shape structure of the scaffold that guarantees ample porosity with appropriate pore size and interconnections in order to permit sufficient transportation and migration of cells, nutrients, metabolites, signal molecules as well as sufficient vascularization to nurture the new tissue growth [12,9]. The concept of bone tissue engineering is to develop and manufacture a biological substitutes (3D scaffolds) that enable the replacing of lost or damage bone caused by disease or trauma [6]. Up to date, bone replacement therapy includes autografts, allografts or xenografts [13,14]. Autografts can be engineered and produced either by culturing autologous cells in vitro guided by a scaffold or by implanting a cellular scaffold in vivo and letting the patient's cells to restore the bone tissue guided by the scaffold. It is preferred that the scaffold degraded at a certain time in harmony with tissue restoration time that means as soon as the tissue has developed the scaffold no longer exists and the newly developed tissue as functional as the lost tissue [15,5]. Such a scaffold must be created from biocompatible material with sufficient physical and mechanical properties as well as having no immunological or clinically foreign body reaction [16,4].

The materials of any scaffold must fulfill the following requirements: “1) biocompatibility 2) sterilizability 3) suitable physical characteristics (mechanical properties) 4) manufacturability 5) biodegradability 6) high porosity with interconnected network of pores and surface chemistry that promotes suitable regulation of cell activities such as a) cell adhesion b) proliferation c) migration and d) differentiation” [17]. There are many substances that have similarity to the bone structures that can be used to manufacture scaffolds to be used for bone tissue regeneration. Of all, natural coral (NC) has been used as a bone graft replacements due to its similar composition of bones. The NC can be transformed into structurally like porous HA via the hydrothermal exchange response. Hence, the mean diameter of the coral pore is 200 μm (190–230 μm) [15].

The basic difference between HA and NC is that the latter is biodegradable and HA is not. Both NC and HA are well known to be osteoconductive, biocompatible and very inert [18,19]. Ever since 1980, natural corallines of calcium carbonate ($CaCO_3$) and its transformed Hydroxyapatite, Ca_{10} $(PO_4)_6(OH)_2$, have been widely used as a replacement biomaterial for bone grafts [15,18]. Moreover, quite a number of biomaterials have been investigated as candidates for bone graft alternatives, including animal bone, chitosan, coral exoskeletons and nacreous materials [2,20]. Best results are obtained with natural biomaterials due to better cell attachment, differentiation and function [21].

The cockle shell consists of about 96% $CaCO_3$ whilst other components include organic substances and other oxides like SiO_2, MgO and SO_3 [22,23]. The $CaCO_3$ has three polymorphs which are the calcite, aragonite and vaterite. Studies have moreover shown that the denser aragonite is an appropriate biomaterial because of its capability to be incorporated and replaced by bone tissues [24–27]. The significant characteristics of an ideal bone implant material are: “i) Biocompatibility ii) Mechanical strength and iii) Safety” [28,6]. The mixture of organic and inorganic resources provides a suitable different choice to mix the best properties of each stage and overcoming a lot of their weaknesses when used as standardized materials [29]. In past 10 years, the cockle shells powder was used to fabricate a novel porous scaffold. The relevance of this substance for manufacturing bone graft replacement comes from previous study conducted by Zuki et al. [22]. The study reported that cockle shells and coral exoskeletons have similar mineral and physicochemical characteristics [22,23,30,33]. Based on this previous study, it was recommended that the cockle shells can be used as good optional biomaterial for bone replacement in organization of bone defects. Gelatin plays a crucial role in the cockle shells research. It has been used widely in tissue engineering scaffolds due to its properties and has biological functions intact as its natural shape collagen such as biocompatibility and physical properties [31]. The aim of this study is to produce, and assess the morphological, physicochemical and mechanical properties of novel aragonite $CaCO_3$ nanoparticles, gelatin, dextran and dextrin derived scaffold as a potential bone matrix for tissue engineering and strengthening material.

2. Materials and methods

2.1. Cockle shells nanoparticles powder preparation

The cockle shells powder was prepared following the method described by Zuki et al., [22]. However, some modifications were made to obtain the best results. The shells were dried in oven (Memmert, UM 500, Germany) at 50 °C for 5–7 days, ground using stainless steel blender (Good and Well®, Taiwan) and sieved through 75 μm sieve (Endecotts Ltd, London, England). The micron sized powder was further dried in the oven (Memmert, UM 500, Germany) at 50 °C for 5 days and kept in air tight polyethylene plastic bag (JP Packaging) until used. The obtained micron sized powder 75 μm was transformed into nanoparticles using a mechano-chemical method in the presence of surfactant BS-12 (dodecyl dimethyl bataine) that was obtained from Sigma-Aldrich (Steinheim, Germany). Briefly, mechanical stirring of the 2 g of 75 μm powder with the 50 mL deionized water (DW) (HPLC-grade of resistance > 18 MΩ obtained from a MilliRO6 plus Milli-Q-Water System (Organex) and 0.5 mL of surfactant BS-12 at 1000 rpm at room temperature for 90 min using the heating homogenize stirrer machine (Wise Stir® Heating Multiple Stirring). The resultant slurry was then filtered using filter paper of size 12.5 cm (Filtres Fioroni, China) and dried at 80 °C overnight then stored at 50 °C in a sterile container prior to use.

2.2. Development of the scaffolds

Three specific natural materials namely gelatin, dextran and dextrin, were mixed together with the cockle shells nanoparticles