Material Properties of a Novel Bio Ceramic Scaffold for the Bone Construction and in Vitro Evaluated Tissue Engineering

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ABSTRACT

In this study, a novel three-dimensional porous scaffold was fabricated from nano particles (CaCO₃) with the micro-macro architecture for the purpose of bone repair, and their material properties were evaluated in vitro. Ideally, scaffold should have the following characteristics: biocompatible and biodegradable, suitable surface chemistry and highly porosity, with an interconnected pore net work. The method may not only accomplish the bone formation on the base of template (scaffold), but also optimize the mechanical properties of new formation. For the in vitro the cells were subculture for 5 weeks on the scaffold. The ability of cells to proliferate on this scaffold was assessed by osteoblasts cells presented a significant increase in alkaline phosphatase activity and calcium deposits were observed at 21 days. Light and scanning electron microscopy revealed the presence of many osteoblast-like cells with development of calcification of the dense collagenous fibril network and bone matrix-like tissue were observed in many area of scaffold, resulting in the formation of bone-like tissue containing osteocyte-like cells. The scaffold properties was characterised by x-ray diffraction (XRD), Fourier-Transform infrared spectroscopy (FT-IR), Scanning Electron Microscopy (SEM), and Compression mechanical tests.

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An introduction is needed for the text to be meaningful. The text seems to be discussing the properties of a novel bio-ceramic scaffold for bone construction and its evaluation in vitro. The introduction should provide context and explain the significance of the research.
compression test of the scaffold was done under the dry condition using universal mechanical testing machine (Instron 4302). The scaffold was also subjected to a 10-day water-soaking test, and examined under high vacuum conditions at 15 KV SEM. FT-IR spectra of calcium carbonate bioceramics scaffold was used to analyses a different materials wave numbers indicated for CaCO₃, gelatin, dextran and dextrin, XRD analysis was done to characterize the crystalline/ amorphous nature of the CaCO₃ and identify any crystalline phases present.

Isolation and Proliferation of Osteoblast Cells

Isolation and proliferation of osteoblast cells from rat bone marrow (RBM) was conducted using the following method. Sprague-Dawley (SD) rats at 6 weeks of age, with an average body weight of 100-200 gram were euthanized using Dolethal, 1ml/kg and the long bones (femur, tibia and humerus) were removed. The diaphyses of the bone were flushed with 10 ml of complete medium after the epiphyses were cut off. The mixture (bone marrow + medium) was resuspended and then 5 ml was transferred into a 75 cm² culture flask filled previously with 10 ml of complete medium. The culture medium used was DMEM (Dulbecco’s modified Eagles medium), supplemented by 10% fetal bovine serum (FBS) and 10U/ml penicillin G, 10 µg/ml streptomycin and 25mg/ml anphotericin B. After 48 hours, the medium was replaced. The medium was changed every 3 days until the cells completely confluenced in the flask. The cells achieved confluence after 10 to 20 days. The MSCs differentiation to the osteoblast cells was achieved by using the differentiation medium. The differentiation medium was used in this experiment (DMEM high glucose). This was supplemented with 10% fetal bovine serum (DNA Company), 50 mg/ mL gentamicin (DNA Company), 0.3 mg/mL anphotericin B (Gibco), 10⁻⁷ M dexamethasone (Merck), 5 mg/l ascorbic acid (Merck) and 7 mM β-glycerophosphate (Sigma). The cells were cultured in a humidified atmosphere of 5 % CO₂ at 37°C.

Cells Seeded onto the Scaffolds

Osteoblast cells were detached using trypsin / EDTA (0.25% w/v trypsin / 0.02% EDTA, Sigma). Sample of the heated scaffold (1cm diameter x 1cm length) were prepared and sterilized. They were then inserted into a 25 cm² flask after pre-wetting with culture medium. Osteoblast cells were seeded with at a cell density of 1 x 10⁸ cells/mL (1 x 10⁷/mL for each scaffold was seeded). The scaffolds were placed in an incubator with 5% CO₂, at 37 °C, and 90% humidity. After 3 hours of attachment, 10 ml of complete medium was added to flask. Seeded scaffolds were incubated for further attachment overnight. The whole volume of medium was changed every 3 days.

IN VITRO EVALUATIONS
Biochemical Analysis (ALP and Calcium)

The alkaline phosphatase activity (ALP) of seeded scaffold and non-seeded scaffold (controls) was assayed as a measure of the osteoblastic expression at 5, 7, 14, 21 and 25 days post-seeding to evaluate the capability of the biosynthetic scaffolds to generate bone in vitro. The alkaline phosphatase activity was measured using a 902 Hitachi automated clinical chemistry analyzer. The calcium (Ca²⁺) content of each scaffold was assayed in order to quantify the amount of
mineralized matrix present. The calcium (Ca\(^{2+}\)) was measured using a 902 Hitachi automated clinical chemistry analyzer.

**Scanning Electron Microscopy (SEM)**

For SEM analysis, sample of scaffold was cut into blocks of ½ cm diameter x ½ cm length. The sample was then fixed in a solution of 4% glutaraldehyde buffer solution at pH 7.3 and 4°C. The coated specimens were observed and analysed using SEM (Tescan VEGA).

**Histological Examination**

For light microscopy, sample was taken from scaffold and fixed in 10% formaldehyde in phosphate-buffered saline (PBS) at pH 7.4 for 24 hours at room temperature. In the standard of histological techniques, immediately after embedding, the sample was blocked with paraffin wax and then sectioned at 5 µm thick. Finally serial sections were stained with:

- Routine Hematoxylin and Eosin for general histology
- Massons trichrome for demonstration of the collagen fiber and new bone tissue.

The slides were examined under a light microscope.

**RESULT and DISCUSSION**

SEM analysis revealed that the scaffold contained of macro-micropores with different sizes. These pores were between 30-380 µm (Figure 1) and showed a uniform interior. The intra-architectural scaffold geometry has a major impact on new bone tissue formation, the physical interpretation of microporosity formed in the scaffold depend on the voids that containing the trapped air can make way to provide space for the swelling effect when the scaffold get wet, and these voids formed between the granules or particles in the all mass material (6). In this study the chemical cross linked for the scaffolds that produced by the heating method, will lead to decrease in the degradation rate. This was demonstrated by the water absorption test that revealed the scaffold lasted for 10 days without much visible surface degradation and water absorption. The scaffold obtained by this method, provide the best compression properties. The scaffold was observed to be uniformly sturdy and strong throughout the test. The degradation rates should be adjustable to match the rate of tissue regeneration (7). The strong and clear peaks of a typical XRD reveal for good crystallinity of obtained product. No characteristic peaks for other impurities could be observed only the major CaCO\(_3\) reflection peak (8) Figure (2). In addition to, the FT-IR of the scaffold revealed no changes could be observed in the spectra of bioceramic harvested at the different steps in the process simulation Figure (3).

Notwithstanding, the in vitro histological examination was considered as one of the most important examinations. The new products of scaffold constructs, which had an initial cell seeding density of 1 × 10\(^8\) cells/cm\(^2\) were sectioned, stained with haematoxylin and eosin (H&E), and counter stained with Massons trichrome for stem cells. The analysis showed the presence of cells and distributed throughout the interior of the 3D- scaffold and also the formation of a thick surface layer of cells Figure (4). Thus, the histological study in this trial was assessed by evaluating qualitatively of the new scaffolds by the interaction between the osteoblasts cells and the scaffold product material. The differences observed in cells proliferation
and continuous to 60 and 90 days after seeding culture, which indicated that multilayer of the cells were observed already accumulated earlier in the outer edges rather than in the middle area of the scaffolds Figure (5). These observations were good in according to Ross and Pawlina (2006) finding. Thus, cell proliferation occurred at different rates throughout the scaffolds. This is likely due to an uneven distribution of cells within the scaffolds during the initial cell-seeding procedure. Initially, more cells anchored to the outer edges than in the center of the scaffolds. The cells were mainly on the surface of the scaffold and few cells inside the scaffold material.

**Figure (1)** SEM microphotograph of scaffold uninfiltrated show the macro and micro porosity (the magnification bars correspond to 1 mm).

**Figure (2)** the graphs show the XRD pattern of the scaffold products. The peak at $26^\circ \theta$ and $28^\circ \theta$ are characteristic of calcium like graphite, and other peaks are characterized of Calcium. This corresponds well to the specification (Graphite, Ca).
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Figure (3) The graphs of FT-IR spectra of calcium carbonate bioceramics; a different materials wave numbers indicated for CaCO$_3$, gelatin, dextran and dextrin.

Figure (4) Microphotographs of the scaffold at 90 days post-seeded stain with Masson’s trichrome show the osteoblasts cells (black double arrows) on the surface of the scaffold material more than the bottom (x 400).
Figures (5) Microphotographs of the scaffold at 60 days post-seeded stain with H&E show the stem cells (double black arrows) on the surface of the scaffold material more than the bottom (x 400).

REFERENCE