Biocompatibility of Porous Alumina-Hydroxyapatite Microcarriers in Stirred Tank Bioreactor for Cell Culture

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Abstract

The biocompatibility study on porous alumina-hydroxyapatite (HA) microcarriers has conducted in a spinner vessel bioreactor using Vero cells. The effect of HA-to-alumina mass ratios on the cell attachment and growth rate of cell was investigated. When HA content in samples was increased, the cells number attached on surface samples increased as well. A good compatibility of the Vero cells to all the porous microcarriers, since the cells were observed already attached at the surface of microcarriers at hours 8 and 120 of incubation time. The cell growth rates of microcarrier containing 0.0, 0.3 and 1.0 w/w were 0.015, 0.019 and 0.017 hour-1, respectively. Carbon content on porous alumina bodies without HA addition was 36.03%, it significantly increased 46.14% when 1.0 w/w HA-to-alumina mass ratio was added.

Keywords: Biocompatibility, alumina, hydroxyapatite, microcarrier, cell culture

Introduction

Standard strategy for treatment of large bone defects is implantation of the bone using autograft, allograft and xenograft (Sopyan *et al,* 2007). Compared to autograph and allograph procedures, use of synthetic materials eliminates problems of donor scarcity, supply limitations, pathogen transfer, and immunity rejection. Thus, the development of artificial bone substitution materials made from metal, polymers and ceramics is a great importance. The ceramics can be used inside the body without rejection due to their biocompatibility, low density, chemical stability and high wear resistance (Abdurrahim and Sopyan, 2008).

Consideration of biomaterials such as porous alumina scaffolds for short- or long–term applications that bring them in contact with body fluids, tissues and organs is not complete without evaluation of their biocompatibility. Cell culture techniques have provided an exceptionally versatile and useful for evaluating aspects of biocompatibility of materials. For bone regeneration and bone tissue engineering applications, an ideal biomaterial scaffold should have the properties of favorable biocompatibility, bioconductivity, and biodegradability. An optimal biomaterial used as a bone substitute should not only be a

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temporary scaffold for supporting the adhesion, growth, proliferation, and differentiation of the 'seed' cells, but also be able to degrade into non-toxic products, which can be via the physiological mechanism (Yaszemski et al, 1996). This paper reports the effect of HA addition on biocompatibility of porous alumina microcarriers fabricated using protein foaming-consolidation method. The microcarriers were applied for cell culture in a spinner vessel bioreactor.

Materials and Methods

Preparation of microcarriers. There were three types of microcarriers has been used for cell cultivation. The microcarriers with spherical shape with diameter of 6 mm were porous pure alumina, porous alumina-hydroxyapatite containing 0.3 and 0.5 w/w hydroxyapatite-to-alumina mass ratios. Before added into spinner vessel, microcarriers were dip into 25% PBS and then autoclaved at 115°C for one hr. Then, 10 samples of each microcarrier were added into each spinner vessel.

Biocompatibility study. VERO cells were used in this work. There were three types of samples with HA-to-alumina mass ratios of 0.0, 0.3 and 1.0 w/w have been used for cell culture test. The samples were formed into spherical shape in 6 mm diameter. Subsequently, the samples were dip into 25% PBS for 1 day and then sterilized in an autoclave for 1 hour at 115°C. The cells were grown in 75 cm2 culture T-flaks at 37°C and 5% CO_2 into 15 mL fresh Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% Fetal Bovine Serum (FBS). After confluence, the cells were detached with 0.05% trypsin.

Figure 1. Vero cell culture in the spinner vessel bioreactor with controlled parameters

DMEM growth media of 190 mL containing 10% FBS was poured into each sterilized bioreactor with 20 samples. 10 percent of obtained inoculums were seeded into each bioreactor and placed on the magnetic stirrer in the incubator with a fixed agitation speed of 35 rpm (Figure 1). Sampling was conducted every twelve hours for 120 hours to determine attached cell concentration on microcarriers. Cell concentration was counted by counting cell released after trypsinization. Specimens were fixed with 4% Glutaraldehyde for 30 min and dehydrated in solutions containing increasing percentages of ethanol (10%, 30%, 50%, 70%, 90%, 100%) before they dried in air oven over night at 37°C. Samples were examined using SEM for the cell morphology analysis.

Calculation of cell viability and specific rate of cell growth, Calculate the percentage of viable cells as follows:

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$$Viable\ Cells\ (\%) = \frac{Total\ number\ of\ viable\ cells}{Total\ number\ of\ cells}$$

The specific growth rate μ (h^{-1}) calculation was estimated by the following equation (Freshney, 2005):

$$\mu = \frac{\ln X_n - \ln X_{n-1}}{t_n - _{n-1}}$$

Where μ corresponds to the value of specific growth rate at any given time point, t (hours) the culture time and X (cells) the value of viable cell cumber for a specific t.

Results and Discussion

In order to evaluate the biocompatibility of porous alumina-HA composites prepared the samples were applied as microcarriers for Vero cells culture in a stirred tank bioreactor. The stirred tank bioreactor was used for cell culture due to its simplicity and ease of monitoring and controlling of growth (Warnock and Al-Rubeai, 2005). Porous alumina samples without HA addition was tested also for comparison. Inoculums for porous alumina samples were 4.8×10^4 cells/mL, whereas for porous alumina-HA composites with 0.3 and 1.0 w/w ratios, 8.6×10^4 cells/mL inoculums were seeded. The cell observation attached on all microcarriers was done every 12 hours. The color changing of media from purple to yellow was observed during the cell culture tests. All media has changed from purple to yellow color due to actively growing cells have produced acidic by-products such as lactic acid that released into the media. It was found that all Vero cells grew well on all samples. The cells with size in the range 20 to 30 µm would attach to the external surface of the alumina-HA composite microcarriers and grow in to the internal pores with 20 to 250 µm diameters, thus being protected from mechanical damage. The cells can break due to fluid dynamic generated stresses, which may arise from agitation and aeration and it is commonly called 'shear damage'. There are three potential damage mechanism to the cells; collision among cell-covered microcarriers; collision with parts of the reactor (especially the impeller); and interaction with turbulent eddies. If the stirring speeds were too high, cells would detach from microcarriers, particularly during mitosis; and if speeds were too low, the microcarriers did not circulate in the medium and cell growth was poor (Ibrahim and Nienow, 2004). Low agitation speed provided the highest Vero cell densities for cultivation of Vero cells on microporous and macroporous microcarriers (Souza *et al*, 2007). Therefore the agitation speed of bioreactor set in these studies was 35 rpm to avoid cell damaged by the impeller speed. Besides the porous alumina-HA composites with mechanical strength in the range 0.2 to 6.4 MPa can reduce mechanical damage of microcarriers by body-body and body-impeller impacts.

Figure 2 shows the attached cells profile that revealed the VERO cell behavior. The number of cells on samples increases with incubation time. After 12 hours cultivation time, the concentration of Vero cell

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attached on porous alumina at ratio of 0.3 w/w ratio and 1.0 w/w porous alumina-HA samples were 4×10^5, 3×10^5, 5×10^5 cells/mL. Increasing the HA content in samples resulted the higher attached cells number. In reality, for porous alumina samples when cultivation time increases from 12 to 24 hours the number cells decrease from 4×10^5 to 1×10^5 cells/mL. It indicated that cells need longer time to adapt to bioinert microcarrier surface, hence number of live cell decreased.

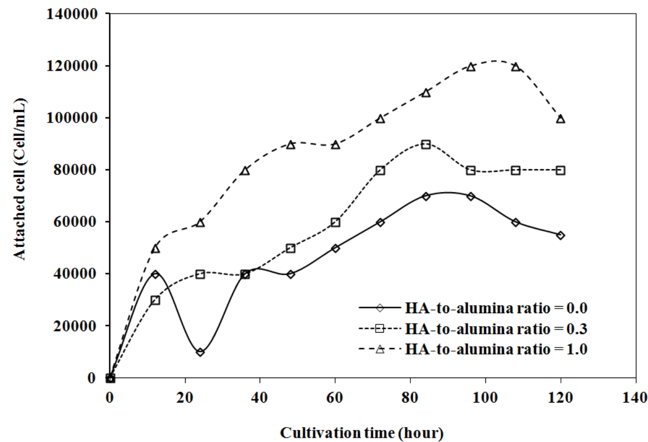

Figure 2. Attached cell profile, showing the biocompatible nature of the samples with different HA-to-alumina mass ratio

After 24 hours cultivated time, the cell number attached on surface of porous alumina-HA was bigger than porous alumina without HA content. Whereas cell number with low HA content was smaller than porous alumina with high concentration. It indicated that HA addition as bioactive ceramics in samples would increase cells number attached on surface samples. Maximum cell number on surface of porous alumina containing 1.0 w/w was observed at 96 h cultivation time.

Data for percentage of viability for each microcarrier was summarized in Table 1. Cells viability was highest in porous alumina culture and lower in 0.3 w/w porous alumina-HA microcarrier. However, the range of viability was more than 50% and it was still suitable for the cells to grow in the bioreactor vessel (Nor *et al*, 2010).

Table 1. Summarized percentage (%) cell viability in bioreactor

	HA-to-alumina mass ratio		
Microcarriers	0.0	0.3	1.0
Percentage viability (%)	77	71	71

Growth rate was useful to determine the performance of Vero cells in the spinner vessel for each porous microcarriers. Specific growth (μ) rate was determined as acceleration phase and microcarrier with 0.3 w/w HA-to-alumina mass ratio has the highest specific growth rate which is 0.019 h^{-1} followed by 1.0 w/w HA-to-alumina mass ratio

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microcarrier (μ = 0.017 h^{-1}) and porous alumina without HA addition (μ = 0.015 h^{-1}). Although microcarrier with 1.0 w/w HA-to-alumina mass ratio obtained the highest attached cells number which is 1.5×10^5 cells/mL, the its cell growth rate was less than 0.3 w/w mass ratio microcarrier. It is indicated that the growth rate of cells was not only influenced by composition of microcarriers but also by surface morphology of the microcarriers. The pore size of 0.3 w/w microcarrier was bigger than 1.0 w/w mass ratio microcarrier.

SEM analysis of cultured cells showed a good compatibility of the Vero cells to all the porous microcarriers, since the cells were observed already attached at the surface of microcarriers at 8 cultured hours. All samples represent a suitable substrate, in terms of adhesion and cell proliferation for Vero cells as the cells adhered well to the microcarriers as confirmed by spreading and growing morphologies.

Figure 3a and b showed that the cells have started to attach and proliferate on porous alumina surfaces at different spaces in 12 cultured hours. The Vero cells attached and spread on surface of microcarriers in a different manner to every sample as shown Figure 3 c. The Vero cell density for pure alumina samples was lower (Figure 4a and b) than the cell density for samples containing 1.0 w/w HA amount (Figure 3c). These studies have supported a strong mutual relationship between attached cell numbers and different microcarrier surfaces. The morphology of cells cultured on porous ceramics was reported to be significantly different than those cultured on smooth surfaces. The discernable differences in morphology of cells can be attributed to the heterogenous topography possessed by the ceramic. The cells tend to attach more intimately with bioactive ceramics such as TCP matrix than alumina (Bose *et al*, 2003). All microcarriers in this work could be observed clearly attached and fully spread. The cultured cell for 120 hours showed an increasing in number of cell-to-cell contacts, establishing further interconnections and evidence of an underlying mitosis process. In this period the cell grows, only if it differentiates and is able to repair itself.

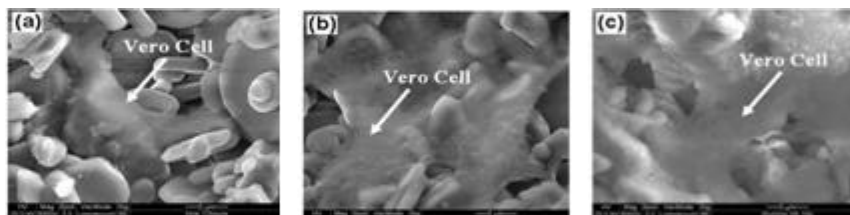

Figure 3. The morphology of VERO cells proliferating on surfaces of the porous alumina for (a) 12 h, (b) 120 h and (c) porous alumina containing 1.0 w/w HA-to-alumina mass ratio, 120 h.

Figure 4 shows the effect of HA content on atomic concentrations of cell attached on surface of porous bodies at cultivation time of 120

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hours. As HA content in bodies increases the carbon atomic amount increases as well. The carbon content on porous alumina bodies was 36.03%, it increased to 37.92% and 46.14% when HA was added 0.3 and 1.00 w/w, respectively. It indicates that HA addition to bodies would enhance cell growth. Carbon, hydrogen, oxygen, nitrogen, sulfur and phosphorus normally make up more than 99% of the mass living cells, and when combined in various ways, form virtually all known organic biomolecules.

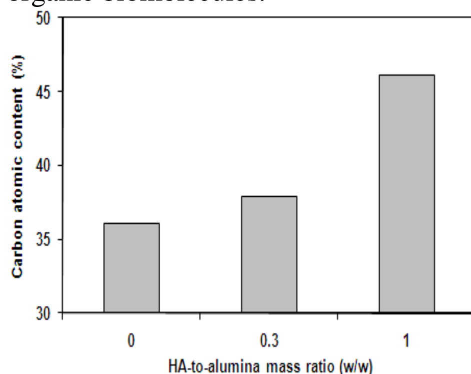

Figure 4. Carbon contents vs HA-to-alumina mass ratio

Conclusion

The biocompatibility of porous alumina-hydroxyapatite composites prepared by protein foaming-consolidation method has evaluated in a spinner vessel bioreactor using Vero cells. The composites with different HA-to-alumina mass ratios (0, 0.3, and 1.0 w/w) were used as microcarriers. The number of attached Vero cells on samples surface increases with the increasing incubation time. The HA addition in samples would increase cells number attached on surface samples. SEM analysis of cultured cells showed a good compatibility of the Vero cells to all the porous microcarriers, since the cells were observed already attached at the surface of microcarriers at hours 8 and 120 of incubation time. The cell growth rates of microcarrier containing 0.0, 0.3 and 1.0 w/w were 0.015, 0.019 and 0.017 hour-1, respectively.

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