Original Article

Biocompatibility Study of a Hydroxyapatite-Alumina and Silicon Carbide Composite Scaffold for Bone Tissue Engineering

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Abstract

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Objective: To date, several scaffolds have been fabricated for application in bone tissue repair. However, there remains a need for synthesis of scaffolds with better mechanical properties, which can be applied to defects in weight-bearing bones. We constructed a composite ceramic bioscaffold of hydroxyapatite-alumina and silicon carbide (HA-Al_2O_3-SiC) to take advantage of the mechanical properties of this combination and show that it supports osteoblast-like cell attachment and growth.

Materials and Methods: Ceramic composite microporous scaffolds were synthesized using an organic template (commercial polyurethane sponge with an open, interconnected microporosity). Osteoblast-like cells (Saos-2) were then cultured on the scaffold and their growth pattern and viability were compared with those cultured in cell culture-treated flasks. Scanning electron microscopy (SEM) was used to assess cell attachment and migration.

Results: The fabricated scaffold shows fairly uniform pore morphologies. Cell growth and viability studies show that the scaffold is able to support osteoblast attachment and growth. However, SEM images indicated that the cells do not spread optimally on the scaffold surfaces.

Conclusion: Our data suggest that that a ceramic hydroxyapatite-alumina and silicon carbide composite scaffold is a viable option for bone tissue repair. However, its surface properties should be optimized to maximise the attachment of osteoblasts.

Keywords: Bone Substitutes, Tissue Scaffolds, Hydroxyapatite, Aluminum Oxide, Silicon Carbide

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Introduction

Implantation of bone autograft or allograft is a known strategy for treatment of large bone defects. However, limited supply, donor site morbidity and risk of transmission of pathological organisms impose major limits to their widespread use. Tissue engineering is trying to address this problem by development of bone substitutes using cells and bioscaffolds. Collagen is the main organic and hydroxyapatite (HA) the main mineral component of the bone extracellular matrix, which determines the mechanical properties of bone and the behaviour of cells. Therefore, these components are used, either alone or in combination, for manufacturing most bone substitutes. To date, several bone substitutes have been approved for clinical applications using a wide range of scaffold materials. However, most of them have relatively poor mechanical strength and they cannot meet the requirements for many applications (1). Hence, there is a need to fabricate new scaffolds with improved mechanical properties and biocompatibility.

In orthopaedic applications, a range of bioactive ceramics such as hydroxyapatite (HA), tricalcium phosphate (TCP), Bioglass and glass ceramics have been employed because of their excellent bioactivity and bone bonding ability (2). Of these, HA ceramic is the most widely used bioscaffold because of its similarity to the mineral component of bone and its good osteoconduction and osteointegration (3). Silica-based ceramics are another group of bioactive products, which exhibit better biodegradability in comparison to HA ceramics, promote

apatite nucleation and enhance bone bonding *in vivo* (4). In addition, silica-based materials encourage deposition of extracellular matrix, which facilitates cell adhesion and other cellular activities (5). Silicon carbide (SiC) ceramic is one of the members of this group which is light weight and has excellent mechanical properties(6, 7). It has been used in the manufacture of composite bone scaffolds, for example with a coating of bioactive glass (8).

Alumina, another material used to make implantable orthopedic devices, is a very well tolerated material with minimum tissue reaction after implantation. It exhibits high mechanical strength and minimum wearing. Therefore, it is frequently used in high load-bearing sites such as hip prostheses and dental implants (9).

Both silicon carbide and alumina support human osteoblast attachment and growth (10, 11). The Saos-2 cell line, which has been derived from an osteogenic sarcoma, has been frequently used as an osteoblast-like cell for biocompatibility studies of scaffolds engineered for bone tissue repair. These cells have shown great potential to adhere and proliferate on anionic gels (12), chitosan scaffolds biomimetically coated with hydroxyapatite (13), and scaffolds with a combination of nano- and microstructures (14). Also, biocompatibility of Saos-2 cells with different scaffold materials, such as polycaprolactone (PCL) (15), have made them potential materials for bone replacement therapies.

We constructed a composite ceramic bioscaffold of HA-Al_2O_3-SiC to take advantage of the mechanical properties of this combination. Here, we show that this scaffold supports osteoblast-like cell (Saos-2) attachment and growth in vitro.

Materials and Methods

The protocols used in this study conform with the ethical codes set by the Ethical Committee of Cellular and Molecular Research Center of Iran University of Medical Sciences.

Ceramic scaffold

The materials used for preparation of the ceramic scaffold were hydroxyapatite (HA) (Merck, Germany), aluminum oxide anhydrous (Merck, Germany), silicon carbide (Carborundum) (BDH, Germany) powders and polyurethane sponge with an open, interconnected microporosity. In this work, we have used the polyurethane sponge as a template for impregnation with HA-Al_2O_3-SiC slurry. Foam impregnation (16, 17) is an established method for the fabrication of highly porous scaffolds with uniform foam morphologies.

Preparation of HA-Al_2O_3-SiC composite

For the preparation of each 100 ml slurry, 40 g HA, 20 g alumina and 10 g silicon carbide (SiC) powders were mixed and milled for 10 hours to provide a powder with an homogenous grain size. The slurry was then made by dissolving this powder in deionized water at a temperature of 45°C using a magnetic stirrer running at 40 RPM for 1 h. Polyurethane sponge was cut into 1-cm cubes and immersed in the slurry. To remove excess slurry the impregnated sponge was compressed between two cylinders and blown at room temperature by a blower. The scaffolds were then dried for 24 hours at room temperature. To eliminate the organic phase (polyurethane), samples were put in a furnace for 6 hours at 800°C. Finally, they were placed at 1700°C for 10 h to sinter the inorganic phase.

Cell Culture

Human osteoblast-like cells, Saos-2 (National Cell Bank of Iran), were maintained in DMEM supplemented with 10% fetal bovine serum (FBS), 500 U/ml penicillin and 200 mg/L streptomycin (all from GIBCO Invitrogen, Germany). Cells were cultured as a monolayer at 37°C in a humidified atmosphere of 5% CO_2/95% air. Cultures were passaged and the cells were harvested by trypsinizing with 1 mM EDTA/0.25% trypsin (w/v) in phosphate buffered saline (PBS) (GIBCO Invitrogen, Germany).

Scaffolds were sterilized for 4 hours at 200°C in an oven, and each side was then exposed to UV light for 2 hours. The sterilized scaffolds were initially rinsed thoroughly three times with PBS and were soaked for 24 h in culture medium. Osteoblast-like cells were then seeded on the scaffolds and the culture medium was changed every other day. Viability of the cells was determined in triplicate samples using trypan blue exclusion before seeding.

Cell counting and growth curve

To determine the scaffold's ability to support growth of the osteoblasts, growth curves for the cells grown on scaffolds were compared with growth curves for the cells grown in tissue culture flasks (Nunc, Germany). After initial seeding, cells from triplicate samples were harvested with 1 mM EDTA/0.25% trypsin (w/v) in PBS at 24 h intervals for 10 days. The average cell count each day was used to depict growth curves.

Cell viability

To compare the viability of cells harvested from scaffolds with those harvested from culture flasks, triplicate samples were assayed using trypan blue exclusion. Cells were seeded on both scaffolds and culture flasks in equal density and harvested by trypsinization one week later. After washing, centrifugation, and adding basal culture medium to the cell pellet, the cell suspension was diluted in 0.4% trypan blue solution (Gibco Invitrogen, Germany) (50:50 v:v) and viable and non-viable cells were counted under an inverted microscope after 3 minutes incubation at room temperature.

Scanning electron microscopy

Cells seeded on scaffolds were harvested for scanning electron microscopy (SEM) after one week. SEM was

performed on both cell-free and cell containing scaffolds. At the end of the culture period, the cell-scaffold constructs were washed with PBS (×3) and fixed in a solution containing 2.5% (v/v) glutaraldehyde (Sigma-Aldrich, Germany) in 0.1 M PBS for 2 hours. Then, the cell-scaffold contructs were soaked in 0.1% (v/v) osmium tetroxide (OSO_4) (Sigma-Aldrich, Germany) in 0.1 M PBS for 30 minutes, and washed again with PBS. Afterward, the constructs were dehydrated in a graded acetone series (30, 50, 75, and 100%) and maintained in 100% acetone until the next step. Finally, the constructs were freeze-dried (BOC Edwards, Crawley, UK) for 6 hours. A scanning electron microscope (SEM, Tescan, PA, USA), fitted with an EDAX energy-dispersive radiograph analyzer, operating at the acceleration voltage of 15 kV was used to visualize the porosity and microstructure of the composites, and osteoblast-like cell growth and attachment to the scaffold. A 10 × 10 × 10 mm slab of the dried scaffold was sputter-coated with gold for 5 min under vacuum at 25 mA before being placed in the SEM chamber.

Energy-dispersive X-ray (EDX) spectroscopy was used for analysis of the elemental composition of target areas for confirming the identity of cells and cellular elements.

Results

SEM-captured images of scaffold morphology show fairly uniform pores, which are suitable for growth of bone tissue cells (Fig 1A, B). Fig 1C shows the grain morphology of the constructed scaffold. The grains are of uniform morphology and their size ranges between 2.5-5 μm.

Cell growth and viability studies showed that the scaffold does not significantly change the behaviour of osteoblasts. Growth curves for Saos-2 cells, Fig 2, show that the population doubling time was 40 ± 0.23 hours for both cells seeded in flasks and on scaffolds.

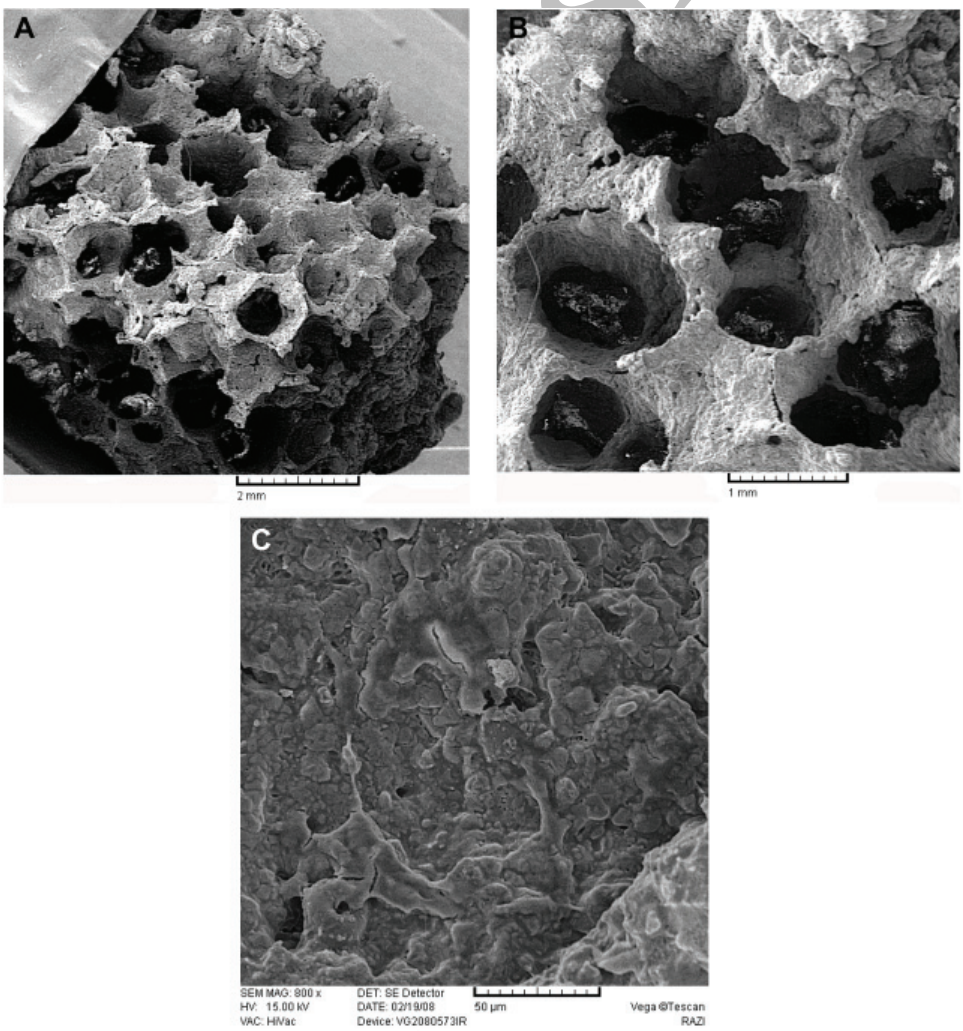

Fig 1: SEM images of the constructed scaffold (A) Low magnification (B) Higher magnification of open interconnected micropores (C) grain morphology.

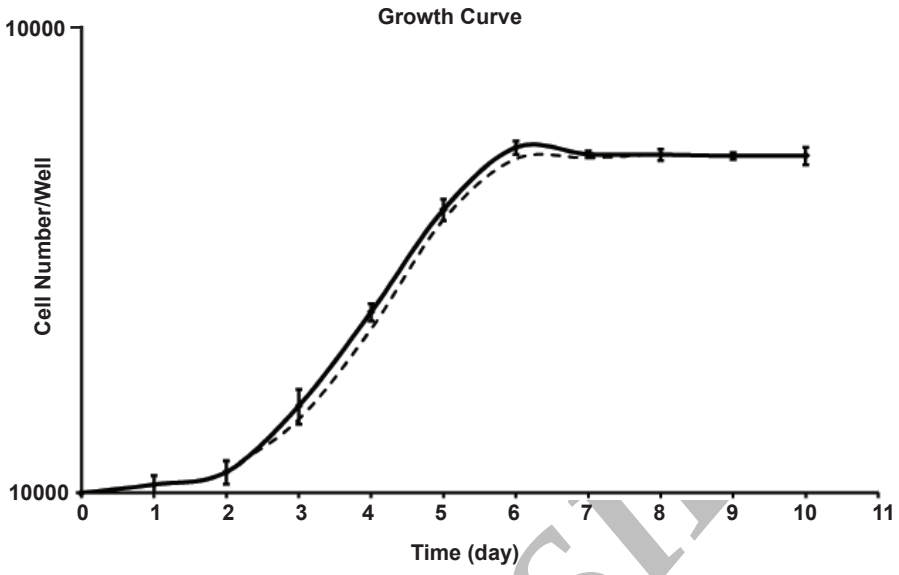

Fig 2: Growth curve of the Saos-2 cell line on both composite (dotted line) and cell culture-treated surfaces solid line. Mean ± SEM (Standard Error of mean) of 3 experiments.

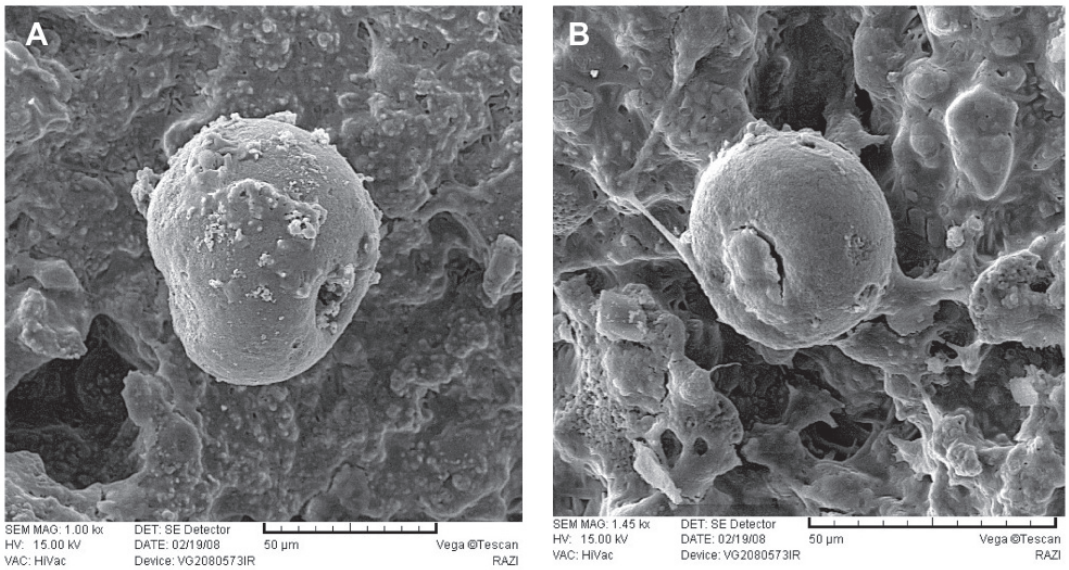

Fig 3: SEM micrographs of osteoblast-like cells attached to HA-alumina-SiC composite surfaces; the cell filaments attaching them to the surface are observed in fig 3B.

Trypan blue exclusion showed that 89% of the cells harvested from scaffolds and 97.5% of the cells harvested from tissue flasks were live.

SEM was used to observe the morphology of the cells and cellular adhesions to the scaffold accompanied by confirmation of cellular identity with EDX spectroscopy. Many cells were attached to the scaffold. However, they did not spread themselves over the surface but rather assumed a homogenous rounded morphology (Fig 3).

Discussion

In this work we constructed a microporous ceramic composite scaffold for application in bone tissue repair. The concept of using bioscaffolds as one of the strategies for tissue repair has been widely accepted, as they can provide structural stability and a 3D system onto which cells can migrate and grow. Bioscaffolds have been synthesized not only for the repair of bone but also for the repair of various other tissues such as cartilage (18), tendon (19), skin (20), blood vessels (21, 22), and the central nervous system (23); and several commercial bioscaffold products are available on the market (24).

Here, we have used a recently developed method involving impregnation of an organic foam for synthesis of a three dimensional scaffold. One of the advantages of this method of construction is the synthesis of scaf-

folds with fairly uniform pore morphologies, which are interconnected and in the range suitable for penetration by osteoblasts and vascular tissue. The pore morphologies we have achieved are comparable to those reported elsewhere (17, 25).

Osteoblast attachment and proliferation were compared on hydroxyapatite (HA) and alumina surfaces. It has been shown that these cells show good adhesion to both surfaces and migrate with formation of filopodia, but they proliferate more rapidly on HA (11). Silicon-containing HA surfaces have also been shown to be suitable for osteoblast adhesion, migration and proliferation. However, decreasing the percentage of silicon, resulted in higher rates of proliferation of these cells (10). In the current work, we have shown it is possible to balance the percentage of silicon with HA and alumina in a way that the rate of osteoblast proliferation remains at the level expected after adhesion to cell culture-treated flasks. However, the morphology of the cells observed by SEM shows that they did not spread and form strong adhesions with the scaffold surfaces. This means that the current composition needs to be optimized by additional measures, such as the incorporation of growth factors or the provision of a surface coating to enable stronger cell adhesions.

Conclusion

In conclusion, we have shown that it is possible to synthesize a highly porous scaffold with materials that confer good mechanical properties using the recently developed organic foam impregnation technique. However, the surface of the fabricated scaffold needs to be optimized to improve the attachment of osteoblasts.

Acknowledgments

The authors declare no conflict of interest in this article.

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