PREPARATION OF CELL-SEEDED HYDROXYAPATITE CERAMIC BEADS

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One of the important ongoing applications of hydroxyapatite (HA) is bone repair. Basically, bone repair using HA is performed by filling bony defects with HA grafts. Ideally, the bony defect was prevented from soft tissue migration by the HA grafting until the end of the new bone forming events at the defect, resulting in bone union and bone repair. Bone formation at the grafted site occurs via recruitment and proliferation of osteoblast precursors, followed, in sequence, by cell differentiation, matrix formation and mineralization. Therefore, the therapeutic result of the HA grafting is highly dependent on the availability of the essential set of cells at the grafting site.

There has been a research topic concerning how to fabricate a perfectly interconnected macropore structure in the HA graft in order to expand the affinity of the HA graft to fluid element in the body that possibly contain essential cells and growth factors. There are several articles reporting HA ceramics with a high macropore connectivity [1]. However, no article confirms the macropore connectivity over the entire HA graft. Further more, even for an HA graft with a perfectly interconnected macropore network, it is highly likely that fluid element affinity of the HA graft decreases at a rate proportional to the size of the HA graft. To load fluid element containing intentional cells over the entire HA graft, there should be some innovation.

We are proposing a new fabrication method of a perfectly interconnected macropore network based on the idea that a porous graft can be built up by gathering tiny HA units. As a model of the HA unit, we have fabricated spherical HA ceramics each with a through-hole (HA beads) [2]. By employing this method, we can provide an HA graft with a perfectly interconnected macropore network consisting of the through-hole and inter-bead gaps. We confirmed remarkable bone formation in the macropore network by a 7-day-long animal test using a rabbit.

Despite the good bone formation, HA itself does not exhibit an active bone forming function. Therefore, the method mentioned above can not fix a settled bone defect such as non-union. Recently, certain cells relating to bone formation are available by ubiquitous cell culture equipment. These cells can possibly be bone forming seeds for the HA graft that can realize an active bone formation. Therefore, HA/Cell hybrids are considered as an advanced mode to establish an effective HA grafting. In this study, we attempted to seed the HA beads by mixing the HA beads and cell suspension and estimated the capability of the HA beads to recruit cultured cells from a cell suspension.

As the HA beads, φ1 mm spherical HA ceramics with a φ 300-µm cylindrical through-hole were prepared in a manner similar to our previous report (Fig. 1). Briefly, HA beads were prepared by sintering φ 1.8 mm gelatinous HA spheres at 1523 K. The gelatinous HA spheres consisted of HA and sodium alginate. Each gelatinous HA sphere was provided with a φ 500µm through-hole obtained by using a trimming tool before sintering. The HA beads were ultrasonic-washed for 15 min and rinsed 10 times with ultra-pure water. The through-hole is a design based on the idea of osteoconductive macro space geometry with respect for the orthopedic application.

As seeds, the mouse calvarial-derived MC3T3-E1 cell line was grown to confluence in alpha minimal essential medium (α-MEM) supplemented with 10% fetal bovine serum and 1% penicillin streptomycin. The grown cells were prepared in a cell suspension (about 1 x 10⁶ cells /ml). The seeding of the HA beads was performed by mixing ten pieces of the dry heat sterilized HA beads and 1 ml of the cell suspension in a 1.5 ml tube using a 500 µl pipettman. The resulting HA beads/cells hybrids in the tube were incubated in an incubator (37°C, 5% CO₂) for 24 hours, then transferred into a 10 cm culture dish containing the culture medium. Twenty-four hours and 7 days after the mixing, cells grown in the HA beads were removed by trypsinization, and estimated by quantitating double-stranded DNA employing fluorescence microplate assay (PcoGreen, Molecular
The HA beads were successfully seeded by the seeding (Fig. 2). The cells removed from 10 HA beads at 24 hours after the mixing indicating an average fluorescence intensity of 198591 counts/sec, thus showing initial cell attachment by the seeding. During the incubation, cells in the HA beads have been grown by forming cell clumps in the through-hole at 7 days after the mixing (Fig. 3). At 7 days after the mixing, the cells removed from 10 HA beads indicated an average fluorescence intensity of 308311 counts/sec. This result demonstrated the superiority of the HA beads to trap suspended cells in a fluid. This result was considered as corroborative evidence to account for the remarkable bone formation in the through-hole during our previous animal test.

The HA/Cell hybrid is maneuverable by conventional tools without damage to the cells in the through-hole. Therefore, the HA/Cell hybrid does not lose its function to seed surroundings after direct manipulations. The HA/Cell hybrid can seed new culture dishes. Furthermore, the HA/Cell hybrid can seed each other, forming a hybrid of HA ceramics and cultured cells. The hybrid was considered as a prototype of the seeded bone graft.

The through-hole holds the potentiality to promote a high-density culture. Growth and proliferation of the cells in the high-density culture was considered to be highly affected by paracrine and juxtacrine factors as well as autocrine factors [3, 4]. In this study, even at 24 hours after the seeding, the through-hole was filled with cells. At 7 days after the seeding, the cells formed an intact clump. Thus, initial packing status of cells in the through-hole was considered favorable for high-density culture to coordinate the valuable actions of autocrine, paracrine and juxtacrine factors.

Consequently, the HA beads has proven to have an adequate properties to utilize cultured cells for bone grafting. The 300-µm through-hole can function as a trap for suspended cells, and as a space for the high-density culture. The seeding of the HA beads was considered an easy and practical method to provide HA/Cell hybrids that would have potential to realize active bone forming orthopedic treatment.

REFERENCES