

Isolation of Amelogenin-Positive Ameloblasts from Rat Mandibular Incisor Enamel Organs by Flow Cytometry and Fluorescence Activated Cell Sorting

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(Accepted 17 November 1997)

The purpose of this study was to use amelogenin as a marker to examine the feasibility of isolating ameloblasts from enamel organ cell populations by fluorescence activated cell sorting. After treating dissected rat enamel organs with proteolytic enzymes to loosen cell attachments and labial connective tissues, dissociated cell suspensions were fixed, then immunostained with rabbit anti-rM179 recombinant amelogenin antibody and FITC-conjugated goat anti-rabbit Ig G antibody. Flow cytometry indicated that about 70% of the total cell sample and virtually all the larger cells therein were amelogenin-positive. Fluorescence activated cell sorting yielded a sample of amelogenin-positive cells at 97% purity. Immunofluorescence microscopy indicated that these isolated amelogenin-positive cells varied widely in size and morphology. This was attributed to loss of intercellular support for ameloblasts once they were dissociated from each other, and to some fragmentation caused when the cells were initially physically removed from the teeth. The results demonstrate that viable ameloblast cell fractions, especially representing cells at the secretory stage, can be purified from enzymic digests of rat enamel organ by sorting on the basis of cell size alone. From these fractions, subpopulations of ameloblasts may be identified when differentiation specific cell surface markers become available.

Keywords: Ameloblast, cell culturing, recombinant amelogenin, antibody, flow cytometry, immunofluorescence microscopy

INTRODUCTION

Ameloblasts differentiate from inner dental epithelial cells to play important roles in enamel formation.^[1-6] Ameloblasts start differentiating during the presecretory stage of amelogenesis under the direction of

growth factor-mediated epithelial-mesenchymal interactions.^[6-17] They then undergo many morphological and functional changes during the secretory and maturation stages, regulated by as yet unknown mechanisms.^[3,5,6,18-20] During the secretory stage, ameloblasts form the whole thickness of enamel by releasing

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Biotechnology Associates, Birmingham, AL) diluted 1:100 with PBS. The cells were repetitively washed with PBS and centrifuged to remove unbound antibodies. Some aliquots of fixed cells were stained with FITC-conjugated goat anti-rabbit IgG antibody alone as a control for binding specificity of the primary antibody.

Flow Cytometric Analysis and Purification of Amelogenin-Positive Ameloblasts

Aliquots of immunostained cells were analyzed with a FACScan® flow cytometer (Becton Dickinson, Mountain View, CA) using Lysys II software. A minimum of 10,000 events were collected per sample. Cell debris and clumps were excluded by setting a gate on forward versus side scatter. Immunofluorescence labeled amelogenin-positive cells from pooled samples were purified using a cell sorter (FACS Vantage®, Becton Dickinson) to collect about 50,000 cells. Aliquots of the sorted samples were examined again by flow cytometry to check their purity.

Immunofluorescence Microscopy

Cells were deposited on glass slides using a cytocentrifuge (Cytospin, Shandon Southern Instruments, Sewickly, PA) run at 1500 rpm for 5 min. A standard epifluorescence microscope (Carl Zeiss Canada, Don Mills, ON) was used for visual analyses.

RESULTS

Isolation of Individual Cells from Enamel Organ and Dental Pulp Tissues

Direct microscopic observations of cell suspensions indicated that large numbers of single cells were released from both enamel organ and dental pulp after enzymatic treatment at optimal conditions (data not shown). Some cell clumps and debris were also found in the suspensions. During subsequent flow cytometric analyses, a gate was set to exclude cell debris (extremely small dimensions) and cell clumps (extremely large dimensions).

Characterization of Amelogenin-Positive Cells by Immunofluorescence Staining and Flow Cytometry

As shown in Figure 1, a large proportion of the dissociated enamel organ cells (about 70%) were positively stained by rabbit anti-rM179 antibody as detected by FITC-conjugated goat anti-rabbit IgG antibody. Their fluorescence intensities were varied, but significantly higher than the remaining cells (Fig. 1) which showed only the same background fluorescence as that of samples stained with FITC-conjugated goat anti-rabbit IgG antibody alone. No anti-rM179 staining was detected among pulp cells (data not shown). Two-dimensional dot plots (cell size axis versus fluorescence intensity) revealed that enamel organ cells varied in size (Figs. 2A and B), particularly among amelogenin-positive cells (Fig. 2B). The maximal difference between large and small cells was about ten times (Figs. 2A and B). Unlike small cells that showed a mixture of positive and negative staining, large cells were almost all positively stained, their fluorescence intensity increasing with increasing cell size (Fig. 2B).

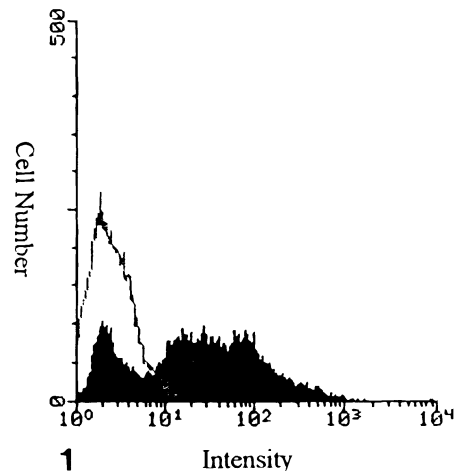


FIGURE 1 Representative flow cytometry histogram showing distribution of enamel organ cells immunostained with anti-rM179 recombinant amelogenin antibody and FITC-conjugated secondary antibody (solid profile, EXPTL, experimental group) or with FITC-conjugated secondary antibody alone (open curve, CONT, control group). The horizontal axis (exponential scale) indicates fluorescence intensity of the cells and the vertical axis represents relative cell counts. Note peaks of amelogenin-positive cells (right) and amelogenin-negative cells (left) in the experimental group.

