One step isolation and staining of CD20 B-cells with quantum dots magnetic beads antibody conjugate from human mononuclear cell cultures for fluorescent microscopy

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Abstract

Aim: Usually it is common practice to isolate the B-cells with magnetic beads in the first step from the mononuclear cell cultures as well as blood samples and in the 2nd step, these isolated cells are stained with different conventional dyes like FITC, PE, Cy3 etc. After these two steps, the cells are observed under fluorescent microscopy. This method takes around 2 hours to be finished. Conventional dyes have problem of cross talking and poor photostability as they fade away quickly.

Quantum dots are nanometer scale semiconductor crystals, which have advantages over the conventional dyes such as they emit the fluorescence in a narrow range, hence there is no cross talking and they have excellent photostability along with excellent fluorescence efficiency. Magnetic beads are being used to isolate different kinds of cells for many years. Therefore, it will be a good idea to use quantum dots magnetic beads antibody conjugate to isolate and stain the cells in one step, which will save time and money. Therefore, in this work, we use CD20 specific conjugate to demonstrate that it is possible to isolate CD20 B-cells with these conjugates from mononuclear cell cultures generated from human buffy coats.

Methods and material: In 10 different experiments, the cells from mononuclear cell cultures were centrifuged to get the pellets. To these pellets, 5 µl of CD20 specific quantum dots magnetic beads antibody conjugate (MICROBOSS Nanomedicine GmbH, Germany) per pellet was added for 15 to 20 minutes. The cells were isolated in magnetic racks, washed and mounted on the slides, which were observed under LED fluorescent microscope with UV filter (Zeiss, Germany). 5 µl of conjugate was added even directly to 500 µl of mononuclear cell culture media to find it is possible to isolate the B-cells without generating pellet.

Results: Isolated cells were observed under normal microscope, which shows that there is isolation of abundant B-cells and magnetic beads are attached on the surfaces of these cells. Under fluorescent microscopy, the cells show specific fluorescence according to the wavelengths of quantum dots 510 nm and 705 nm. The whole procedure finished within 30 minutes against 2 hours with two steps.

The method was also successful with directly adding the conjugate to cell culture without generating pellet.

In both cases, there were only isolation of CD20 positive B-cells without other cells.

Conclusion: This should be one of first report in literature about the successful use of one step isolation and staining of CD20 specific B-cells from human mononuclear cell cultures for fluorescent microscopy within 30 minutes against the two steps method isolation of cells, which may need around 2 hours. This method provides cells with brighter fluorescence against the present methods with conventional dyes. This work can save money and time.