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Application of retrograde dissection method for isolation of bone marrow cells from rat femurs and tibiae

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ABSTRACT. Currently, there is no practical and efficient method for the isolation of bone marrow cells (BMCs) from rat femurs and tibiae. Here, we attempted to develop a rapid, simple, effective, and non-contaminating method for the isolation of BMCs from rat femurs and tibiae. Rat femurs and tibiae were dissected from the ankle to the hip joint; subsequently, a three-step "locate-slide-twist" procedure was performed using scissors and forceps to remove the femurs and tibiae completely, from the surrounding musculature. The bones were flushed with phosphate-buffered saline to harvest BMCs. The femurs and tibiae were dissected in 1.8 ± 0.6 min, and the BMC suspension preparation time was 13.1 ± 2.3 min. The bone marrow cavities did not incur any fractures or injuries during the isolation. Culture of harvested BMCs for 72 h led to a significant increase in cell number from 4.4 ± 0.3 x 10^6 to 6.9 ± 0.7 x 10^6 ($P < 0.01$) with no significant decrease in viability (98.1 ± 0.6% *vs* 96.2 ± 1.1%; $P > 0.05$). Microscopic examination of the isolated BMCs after the 72-h incubation period revealed the no-microbial or muscle cell contamination. Furthermore, flow cytometry revealed that cultured BMCs (72-h culture)

grew well. Here, we have reported a rapid, simple, effective, and non-contaminating method for the isolation of BMCs from rat femurs and tibiae by using retrograde dissection. This method can be used to harvest a large number of viable BMCs without the risk of contamination from muscle and connective tissues.

Key words: Retrograde dissection; Tibiae; Femurs; Rat; Bone marrow cells

