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OPTIMISATION OF CULTURE AND CARADIOGENIC DIFFERENTIATION OF RATS MESENCHYMAL STEM CELLS*Chang Jing Cardiology Department, First Affiliated Hospital Of Chongqing Medical University, Chongqing, China*

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There has been an increasing interest in mesenchymal stem cells (MSCs) because of their potential use for regenerative therapy, especially for cardiovascular disease, however there is still no well-defined protocol for culture and induction of cardiogenic differentiation for them. In this study, different conditions for the isolation, expansion and induction of cardiac differentiation of rat MSCs were tested. When plated at the density of $10^8/\text{cm}^2$ for 72 h, the primary culture was predominated by recycling stem cells (RS cells), and the characteristics of the rat MSCs (including morphology, growth rate, phenotype and differentiating potentials) were kept stable during the expansion until the end of observation. Compared with traditional plate-culture, culture with gelatine porous microcarriers Cultispher-S was superior for the large-scale production of rat MSCs. Rat MSCs could be induced to differentiate along cardiogenic lineage by combined treatment of 5-azacytidine (5-aza), retinoic acid (RA) and sulfoxide (DMSO) in middle-dose, as well as by the treatment of coculture or conditional medium of neonatal rat myocardium, but

not by the treatment of 5-aza alone, combined treatment of 5-aza, RA and DMSO in low-dose and high-dose, or by supernatant of neonatal rat myocardium. These results suggest that plating density and plating time in primary culture played an important role in the building of immortal culture system, microcarriers Cultispher-S could be an attractive candidate for large-scale production of MSCs, and the mechanism of cardiogenic differentiation should be further explored for a better control of this process.