CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

MSCs were initially isolated from bone marrow by Friendenstein *et al* (Friendenstein *et al.,* 1976) and then studied by other investigators (Caplan *et al*., 1992; Clark *et al*., 1995; Bruder *et al*., 1997; Zohar *et al*., 1997; Pittenger *et al*., 1999). Recently, MSCs have been found to have the potential to differentiate into muscle cells, adipocytes, osteocytes, and chondrocytes in culture (Pittenger *et al*., 1999; Lenon *et al*., 2001; Sekiya *et al*., 2002). After systematic injection, MSCs are incorporated into a variety of tissues, including bone (Pereira *et al*., 1995), muscle (Ferrari *et al*., 1998), lung (Pereira *et al*., 1995), and epithelium (Spees *et al*.,2003). MSCs from bone marrow have also been shown to form cardiomyocytes (Fukuda *et al*., 2001; Orlic *et al*., 2001; Alvarez *et al*., 2003). Therefore, the expected potential of MSCs is a matter of paramount for upcoming therapeutic strategies in the context of cellular therapy. Although the biological characteristics of MSCs have been subjected to intense investigation over the past few years, there is presently no universal accepted definition of this type of stem cell. Despite the lack of a definition, BM-MSCs have already been used as the main source in various preclinical and clinical studies (Pereira *et al*., 1995; Horwitz *et al*., 1999; Koc *et al*., 2000). However, the use of adult bone marrow has some limitations. First, the frequency of MSCs in adult bone marrow is very low (0.001%-0.01% of total cell population). Second, the number and proliferative capacity of BM-MSCs are significant decrease with age (Mueller *et al*., 2001; Stenderup *et al*., 2003). Moreover, harvesting bone marrow from a patient is an invasive procedure. Therefore, the search for alternative sources of MSCs for experimental and therapeutic application is important.

This study demonstrated that umbilical cord, Wharton' jelly, placenta and amnion are the alternative sources of MSCs. UC-MSCs, WJ-MSCs, PL-MSCs and AM-MSCs could be isolated from postnatal tissues through mechanical and enzymatic digestion as previously reported (Romanov *et al*., 2004; Wang *et al*., 2004; Lu *et al*., 2006; Barlow *et al*., 2008; Bilic *et al*., 2008). They shared most of the characteristics with BM-MSCs (Antonitsis *et al.,* 2007; Jiang *et al.,* 2007;

Khoo *et al.,* 2008; Terada *et al.,* 2002; Wagner *et al.,* 2005), including fibroblast-like morphology, typical immunophenotypic markers, adipogenic and osteogenic differentiation capacity as well as immunomodulatory properties. However, several differences were observed in this study. Firstly, the proliferative capacity of UC-MSCs and WJ-MSCs, were significantly higher than that of BM-MSCs ($P<0.05$). In contrast, PL-MSCs and AM-MSCs, have significantly lower proliferative capacity than that of BM-MSCs ($P<0.05$). These results indicate a higher proliferative capacity of UC-MSCs and WJ-MSCs in comparison to BM-MSCs. However, the mechanisms underlying these differences are unknown. The further study in cell-cycle regulation of those MSCs from different sources may prove beneficial in answering that question.

Flow cytometry indicated that the immunophenotype of UC-MSCs, WJ-MSCs, PL-MSCs and AM-MSCs is similar to that of BM-MSCs which highly expressed several MSC markers including matrix receptor (CD105) and integrin markers (CD73, CD90) but did not express hematopoietic markers (CD34, CD45). These results suggested that the immunophenotypic characteristic of UC-MSCs, WJ-MSCs, PL-MSCs and AM-MSCs are not different from that of BM-MSCs. (Pereira *et al.,* 2008; Mitchell *et al.,* 2003; Lu *et al*., 2006; Fukuchi *et al.,* 2004; Miao *et al.,* 2006)

The multi-lineage differentiation capacity of UC-MSCs, WJ-MSCs, PL-MSCs and AM-MSCs were study through the osteogenic and adipogenic differentiation as previously reported (Pereira *et al.,* 2008; Mitchell *et al.,* 2003; Seshareddy *et al.* ,2003; Fukuchi *et al.,* 2004; Miao *et al.,* 2006; Alvino *et al.,* 2007; Toda *et al.,* 2007). Similar to BM-MSCs; UC-MSCs, WJ-MSCs, PL-MSCs and AM-MSCs were capable of differentiating toward osteogenic and adipogenic lineages. However, in contrast to BM-MSCs; it took a longer period of time for PL-MSCs and AM-MSCs to differentiaten to adipogenic lineage. Several publications have shown that BM-MSCs are heterogeneous cell populations and that in multipotent clones the adipogenic potential is the first to be repressed (Muraglia *et al.,* 2000). On the other hand, the difference in the sensitivity to undergo adipogenic differentiation may also represent an inherent difference between sources of MSCs. Because adipocytes, which present in adult bone marrow, are absent in fetal bone marrow. This suggests that the potential of MSCs

to become adipocytes may increase with age (Gimble *et al.,* 1996). Therefore, the lower sensitivity of PL-MSCs and AM-MSCs compared to that of BM-MSCs may be related to the lower age of their tissues of origin.

MSCs have the potential to reconstitute bone marrow stroma and produce cytokines which may speed up hematopoietic reconstitution (Verfaillie *et al.,* 1998; Arroyo *et al.,* 1999). Another unique characteristic of MSCs is their ability to induce allograft tolerance (Bartholomew *et al.,* 2002; Le *et al.,* 2004). There is considerable interest in using MSCs in hematopoietic stem cell transplantation. This study also investigated whether UC-MSCs, WJ-MSCs, PL-MSCs and AM-MSCs have the similar immunomodulatory capacity as that of BM-MSCs. The result supports the previous reports on the immunosuppressive properties of BM-MSCs (Le *et al.,* 2004; Di *et al.,* 2002; Aggarwal *et al.,* 2005). In addition, our result showed that PL-MSCs and AM-MSCs exhibited stronger immunosuppressive effect than that of BM-MSCs, at least in terms of the ability to suppress an alloreactive T-lymphocyte in MLR assay. In contrast, UC-MSCs and WJ-MSCs exhibited lower immunosuppressive effect than that of BM-MSCs. However, those differences in immunosuppressive effect are not statistically significance. These findings indicating that UC-MSCs, WJ-MSCs, PL-MSCs and AM-MSCs could be used to treat corticosteroid-refractory acute graft versus host disease (Ringden *et al.,* 2006; Fang *et al.,* 2006)

However, the mechanisms underlying the immunosuppressive effect of MSCs are not well defined. At present, it is still not known whether MSCs suppress T-cell proliferation through direct cell-cell contact, or through the production of soluble factor(s) such as human leukocyte antigen-G (HLA-G), interleukin-10 (IL-10), leukemia inhibitory factor (LIF), indoleamine oxidase (IDO), and transforming growth factor- β (TGF-β) (Di Nicola *et al.,* 2002; Djouad *et al.,* 2003; Beyth *et al.,* 2005; Nasef *et al.,* 2007). The investigation of mechanisms underlying the immunosuppressive effect of MSCs may improve the use of MSCs in clinical application

One of the most important benefits of using UC-MSCs, WJ-MSCs, PL-MSCs and AM-MSCs for clinical application are the availability. Moreover, it is possible to obtain umbilical cord, Wharton's jelly, placenta, amnion and umbilical cord blood (UCB) from the same donor. Therefore, UC-MSCs, WJ-MSCs, PL-

MSCs and AM-MSCs are attractive sources of MSCs for co-transplantation in conjunction with UCB-derived HSCs (In *et al.,* 2003; Noort *et al.,* 2002).

In conclusion, this is the first study to compare the characteristics of MSCs from 5 sources, UC-MSCs, WJ-MSCs, PL-MSCs, AM-MSCs and BM-MSCs. The results obtained from this study demonstrated that umbilical cord, Wharton's jelly, placenta and amnion are the rich sources of MSCs that can be easily expanded in culture. UC-MSCs, WJ-MSCs, PL-MSCs, AM-MSCs and BM-MSCs are similar in terms of growth requirements and biological properties. UC-MSCs and WJ-MSCs have higher proliferative capacity than BM-MSCs under the same culture conditions. Moreover, UC-MSCs, WJ-MSCs, PL-MSCs, AM-MSCs and BM-MSCs also displayed a similar degree of immunosuppressive capability in MLR assay. Taken together, UC-MSCs, WJ-MSCs, PL-MSCs and AM-MSCs could provide a novel and non-invasive sources of MSCs for future clinical applications.