spaces between those tubular structures. Nonetheless, the in vitro reconstruction appeared comparable to that reconstructed in vivo, ectopically in subcutaneous spaces of mice, observed during the previous study [7]. This means that the microenvironment in a body is not necessary for the reconstruction of those testicular cells. One of the significant differences between in vivo and in vitro microenvironmental conditions would be the presence of microcirculatory systems. In fact, the presence of a microcirculatory system is mandatory for maintaining the homeostatic function of each organ and tissue. Based on the present results, however, we can argue that such microcirculatory systems, such as capillaries and lymph vessels, are not necessary for the reorganization of testicular cells. Nonetheless, it is also possible that further refinement of the reconstruction would need the microcirculatory systems.

On the other hand, we were not able to identify germ cell differentiation beyond the meiotic phase. This limitation may not be in vitro-specific, because it was also recognized in previous work involving ectopic reconstruction of the testicular structure in the subcutis [7]. The incomplete structure of the reconstructed tubules along with the unbalanced composition of each testicular somatic cell type, including Leydig and peritubular myoid cells, might be its cause. It was reported that the deformity of seminiferous tubules and lack of adult-type Leydig cells in desert hedgehog null mice are relevant to its spermatogenic failure [22]. The proper maturation of testicular somatic cells, especially Sertoli and Leydig cells, is mandatory for the normal progression of spermatogenesis. The cell dissociation and subsequent incomplete reorganization of the testicular structure could impair the maturation of those cells. Immature Sertoli cells in particular may not be able to build the blood-testis barrier, which plays an important role in the proper progression of spermatogenesis [23].

Another point that needs improvement is the efficiency of germ cell incorporation into the reconstructed tubules. To induce spermatogenesis over a larger area of the reconstructed tissues, germ or GS cells have to be incorporated effectively in the aggregates of Sertoli cells, because their aggregation is the initial step of tubular formation. During gonadogenesis in the embryo, primordial germ cells appear to have affinity with precursors of Sertoli cells. Such an affinity did not seem to be shared with the interaction between GS cells and neonatal Sertoli cells. For more efficient spermatogenesis in the reconstructed tubules, certain procedures may be needed to induce such an affinity without disturbing the tubular formation.

Our in vitro reconstruction method reliably established the initial phase of spermatogenesis and is easy to perform, so it will be a useful method for the study of testicular organogenesis and spermatogenesis. The combination of cells from different sources, not only germ cells but also different kinds of somatic cells, will be possible for testis reconstruction. When those cells become available from induced pluripotent stem (iPS) cells or other accessible cell sources [24, 25], testicular tissue fragments could be rebuilt in vitro from those cells without depending on the testis as a cell source in the future, which will make this method more useful and even practical for application to the study of human spermatogenesis.

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