論 文 の 内 容 の 要 旨

論文題目 Application of endometrial stromal cells in engineered tissue constructs to promote uterine regeneration and early implantation of embryo
（子宮再生および胚の初期着床を促進するための組織形成における子宮内膜細胞の適用）

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Uterus is an essential organ for embryo/fetus to grow until women give birth. Recently, the rate of uterine diseases has been tremendously increased, further causing decrease in pregnancy rate. When the uterine diseases such as gynecological cancer, asherman's syndrome, adenomyosis, etc, become severe, most of the patients are subjected to hysterectomy that refers to a removal of uterus with the defects or malignant tumors. This surgical procedure, however, results in permanent infertility from the patients. On the other hand, while the IVF has highlighted for the patients who are suffering from infertility, it still remains several issues. When the assisted hatching was performed during IVF procedure, it sometime damages the fertilized zygote, or the zygote sometimes degenerated *in vitro* while the zygote is usually required to be cultured on the culture dish for its development for 2 – 6 days during the IVF. Moreover, one of the main reasons in the IVF failure refers to the low success rate of implantation onto the endometrium after the cultured embryo is transferred to the patient. Hence, those issues left in the techniques of IVF are required to be solved in order to promote its success rate and further to overcome the infertility.

In order to solve aforementioned issues, tissue engineering approaches have been discussed by many researchers. For uterine regeneration, our group has attempted to utilize

decellularized scaffold that is fabricated by high hydrostatic pressure. We showed that the decellularized scaffold helped the uterine tissue in murine model regenerated after 1 month of transplantation, but the total uterine regeneration was not completely achieved particularly in the smooth muscle layer. The motivation of this research was to find out a new model for uterine regeneration.

The uterus consists of two layers involving the inner layer of endometrium and the outer layer of myometrium. The endometrium is subjected to a dynamics condition due to the contractile movement of the myometrium. Mechanical stimuli in the uterus are regarded as a significant factor for its physiological function on a par with biochemical stimuli induced by estradiol and progesterone. However, its effect still remains unknown. In the first study of this dissertation, we studied the effect of cyclic strain on the human endometrial stromal cells (hESCs). We firstly find out a new finding that applying cyclic strain onto hESCs for 7 days significantly up-regulated uterine smooth muscle cell (SMC) markers. The up-regulation of SMC markers in hESCs in response to cyclic strain was accompanied with increase in the cAMP production level. In order to determine the importance of cAMP in the stretch-induced differentiation, we carried out inhibitor tests using the adenylyl cyclase inhibitor SQ22536 and the PKA inhibitor H-89. As a result of the inhibitor tests, we revealed that this phenomenon in hESCs in response to cyclic strain was occurred via cAMP signaling pathway. The change in hESCs against mechanical stimuli may imply the further possibility of hESCs as new cell source for uterine regeneration particularly in the outer layer of uterus that mainly consists of smooth muscle layer (myometrium). The cell contraction assay using collagen gel was undertaken to prove its innate ability of contraction like uterine smooth muscle cells (SMCs). The strained samples for 7 days showed the promoted contractile ability compared to the control samples. In addition, the strained cells were further contracted by addition of oxytocin, which is known to regulate the contractility ability of the myometrium in vivo. Since the endometrium is exposed to dynamic condition induced by the myometrium, ESCs together with dynamic condition may contribute to the differentiation of SMCs in the uterus and become a source of SMCs *in vivo*. It further highlights an importance of dynamic condition for ESCs within uterus while ESCs are known to play a significant role in wound contraction for the self-repair of uterus. These results suggested that hESCs have a potential possibility in applications for three-dimensional uterine reconstruction particularly strengthening the myometrium.

In the second study, we attempted to develop a new model using endometrial stromal cells in order to examine its *in vivo* application for uterine regeneration. We developed a new method to reconstruct the SFT using a rESC monolayer formed underneath the bottom of the SFT, so

that it allowed to retain its original shape as expected. The reconstructed SFT *in vitro* was transplanted to 9 weeks old female SD rats. Since the rESC SFT did not contain any artificial materials, it was regarded as a great *in vivo* model to reveal the uterine regenerative mechanism. Throughout the time-dependent transplantation experiment, we found out that the epithelial cells from the native tissue started covering the SFT. It was integrated into the endometrium in as little as 3 days after transplantation. The SFT transplanted after 14 days showed complete regeneration and was spread out entirely in the endometrium without any inflammation. We showed that the potential possibility of the SFT using endometrial stromal cells for uterine regeneration.

The last study involves evaluation of in vitro application of SFT reconstructed by hESCs in order to induce early implantation of rat embryo. While hESC contains such a strong cell-cell interaction force that makes difficult to retain its shape in a certain three-dimensional structure, we established a novel method to fabricate the tissue-engineered constructs using ROCK inhibitor without other mediator such as artificial materials or scaffolds. This three-dimensional disc shape of tissue-engineered constructs using hESCs provided a superior environment *in vitro* for rat embryo to induce its early implantation of the embryo in terms of hatching, development, and attachment. As compared to the monolayer condition of hESC, the hESC SFT had a great increase in specific gene expressions (i.g. IGFBP1, COX2, LIF, and STAT3) that are known to highly secrete *in vivo* during blastocyst implantation. We first took notice of the significance role for the use of hESC in the form of three-dimensional structure as a new *in vitro* model.

In this dissertation, we elucidated the effect of mechanical stimuli on the endometrial stromal cells for uterine tissue engineering. Moreover, a new model for tissue engineered constructs has developed using ESCs. The data combined delivered new insights for the usefulness of mechanical stimuli as well as tissue engineered constructs in order to fulfill uterine regeneration with the tissue engineering approaches. Particularly, newly *in vitro* and *in vivo* strategies of the tissue engineered constructs, scaffold-free tissue, will be expected to solve the current issues facing women who are suffered from the infertility. As exerting a superior environment for embryo, the scaffold-free tissue using endometrial stromal cells can be utilized as new concept of an incubator for embryo during *in vitro* fertilization to retain the health condition of embryo or to accelerate development of embryo. In addition, coculturing embryo on the SFT can provide the three dimensional situation mimicking the situation that the embryo attaches onto the endometrium during early implantation in pregnancy, compared to the conventional experimental model using

monolayer of ESCs. By using this three-dimensional SFT model, it can also utilized as *in vitro* model to reveal the molecular mechanism in order to find out key factors to regulate early implantation during pregnancy. This finding will be a breakthrough to contribute to a fundamental treatment of any deficiency in any of those essential genes during early implantation of the embryo. Lastly, the SFT can be utilized to develop a uterine implantation patch. The concept of uterine implantation patch is to implant the SFT as well as the embryo *in vitro*. by coculturing embryo onto the SFT until the embryo become attached within 1 – 2 days, the SFT with the embryo attached can be transplanted altogether to the endometrium. The conventional method of IVF waits for the embryo to attach onto the endometrium *in vivo*, so that it results in very low rate of success pregnancy rate due to the low implantation rate of the embryo. Hereby, our proposal makes the embryo to attach the SFT *in vitro* prior to transplantation. After the attachment of embryo onto the SFT is confirmed, the SFT as well as embryo attached will be transplanted to the endometrium. This new concept of uterine implantation patch will be expected to overcome the low success rate of pregnancy, further playing a role to overcome the female infertility.