Organogenesis and tissue regeneration of fallopian tube: A desired metaplastic transformation of mesodermal stem cells in live animal models (dogs) 

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The capacity of stem cells of peritonium of mesodermal origin to undergo metaplastic transformation and form different tissues developed from mesoderm germ layer is exploited with ulterior motive to use it in the management of human diseases. The excised fallopian tube was replaced with a tube on a stem constructed from autogenous peritoneum from a suitable donor site. The effect of the surroundings environment of the new tissue system to which the peritoneum stem cells are now exposed was studied for 3, 6 and 12 months period in live animal models. The gross and histological studies revealed development of all the component of the wall of the fallopian tube. The lumen of the constructed peritoneal tube was well preserved in its whole length including the anastomotic sites. The scientific rationale of the working hypothesis on which the work is based, is discussed.

Plastic surgery of fallopian tube has witnessed use of variety of tissues indicating interest in the area of infertility in females. Despite varied success rates and notable improvement in over all results in their use in plastic surgery of the tube, these substitutes were not integrated in the process of natural wound healing and repair. The suitable method to replace the part or whole of the fallopian tube is still needed to correct the extensive damage of the fallopian tube. The regenerative technique to grow tissues and organs in the body is probably the most ideal and desired venture to accomplish. The pluripotent stem cells present in the tissues of the body are responsible for healing and repair of tissue in the damaged or injured region. But when exposed to irritation or abnormal conditions they have the capacity to undergo metaplasia. This transformation is often abnormal / pathological in the region. If stem cells in the process of differentiation and proliferation to replace morphologically different tissue are subjected to undergo change which is desired in the region of new

With the capacity of the peritoneum of mesodermal origin to undergo metaplastic transformation and form different tissues developed from mesoderm germ layer, the peritoneal tube was replaced with a tube on a stem constructed from autogenous peritoneum from a suitable donor site. In the surroundings environment then the desired metaplasia may be responsible for tissue/organ regeneration. Accordingly a hypothesis was proposed, based on the success and failure of experimental studies in experimental live animals. The present study has been undertaken to:

(A) Test Matapurkar’s hypothesis 

(A) Regenerate fallopian tube in vivo in dogs.

The hypothesis states that if stem cells of the autogenous peritoneum from the embryonal contiguous region to urogenital tract are exposed in vivo to the surroundings and the environment of urogenital tract tissue system, then these pluripotent cells may differentiate and proliferate into desired tissues in the region based on the principles of embryonal organogenesis.

Materials and Methods

All the norms laid down by the local ethical committee for the conduction of the experiments on animals were strictly followed. The experiments were attempted under general anesthesia in 7 female Mongrel dogs of 4-8 years of age having 7-10 kg body weight. The fallopian tube segment measuring 5-7 cm size was excised (Fig. 1A and C) and replaced by a free peritoneal tube. The tube was constructed from the donor peritoneum measuring 2-8 cm. excised
from retro vesicular pelvic and posterior abdominal wall peritoneum. By suturing the edges of excised peritoneum with 5/0 Vicryl eye less suture on a suitable sized infant feeding tube. The stent bearing the peritoneal tube was transferred to excised fallopian tube region. The ends of the stent introduced into the cut ends fimbrial and uterine ends of the fallopian tube. The uterine end of the stent tube was brought down through uterus into vagina and secured with the wall of the vagina in its lower part, with a non absorbable suture, to avoid accidental slip (Fig. 1B). The peritoneal tube was anastomosed with the spatulated cut ends of the fallopian tube to avoid stricture formation at the site of the anastomosis. The stent was removed after 3 months of post operative period. The biopsy specimen were obtained after observation period of 3 months (3 dogs), 6 months (3 dogs) and 12 months (1 dog) after re-exploration under general anesthesia. After studying the gross changes in and around the graft region, the grafted peritoneal tube along with normal fallopian tube beyond the site of the anastomosis, was excised. The patency of the grafted tube was confirmed by injecting saline through one end of the excised specimen and observing the free flow of the saline at the other end. The microsections and the staining procedures were followed as per standards of the Institute of Pathology, Indian Council of Medical Research, New Delhi. The histology was studied in Eosin and Haematoxyline and Masson's Tri chrome stain.

Results

The post operative period in all the 7 dogs was uneventful except in one dog where the stent was pulled out accidentally on 3rd post operative day. There was some bleeding per vaginum in this dog which stopped by itself without any intervention.

Gross examination: On re-exploration under general anesthesia at 3 months postoperative period, the grafted site revealed easily separable flimsy adhesions around the graft. The grafted tube showed definite change in coloration from pale yellowish (Fig. 2A) to pink and rosy red (Fig. 2B). The tube was thickened and turgid. It was comparable to the normal fallopian tube texture. The mucosal surface was smooth and shining. The transverse section of the peritoneal tube and the graft turned fallopian tube were similar and comparable having well preserved lumen and thickening of the wall of the graft (Fig. 2B). At 6 and 12 months period the adhesions were more firm but separable by blunt dissection or occasionally by sharp dissection (Fig. 2C). In one dog where the stent was accidentally dislodged on 3rd postoperative day, the tubular configuration was lost near the uterine end, but rest of the graft maintained tubular structure of the graft. The uterine opening of the fallopian tube was blocked. In all other dogs the tubular structure of the graft was well preserved and maintained. The tube was patent as evidenced by the free flow of the saline injected from the fimbrial end of the fallopian tube into uterus. There was no stricture formation at the anastomosis sites of the graft with the fallopian tube.
Microscopic examination: H & E Stain: The histology of the graft turned fallopian tube (Fig. 3a, 6.3 x10, magnification) was comparable to the normal fallopian tube (Fig. 3b, 10x10 magnification). The components of the wall developed were in the same order as normal. The 10x25 magnification revealed mucosa, sub mucosa, muscularis mucosa and serosa (Fig. 3e). All the components of the wall of the fallopian tube in the region of the grafted peritoneal tube were well developed. There was chronic inflammatory cell infiltration in the mucosal and serosal layer at some places in 6 out of 7 cases. The smooth muscles were arranged in two distinct layers. The cilia were not demonstrated in 6 out of 7 cases. In one dog where oestrogen was used before taking biopsy specimen the cilia could be demonstrated (Fig. 3d, 10x40 magnification). The Masson's trichrome stain revealed development of connective tissue in green and muscle tissue in pink colour (Fig. 3e).

**Discussion**

The single celled ovum once fertilized acquires a tremendous capacity to form tissues and organs of the whole body having different structures and functions. How this is triggered was probably known during ancient period*. This task is achieved by the totipotent cells of the fertilized ovum and developing embryo. On specialization these cells form germ layer cells of the ecto, meso and endoderm of germ disc of the embryo (Fig. 4a). In this process of specialization the toti potency is reduced to pluri potency, that is, now these cells can form only a few tissues that are developed from ecto, endo or mesoderm. All the body tissues are formed by single germ layer or by the combination of more than one germ layer. Once these cells get specialized, they lose reversibility to pluri...
Fig. 3—Microscopic examination: Histology (H & E Stain): (a) Graft turned fallopian tube 6.3x10 magnification at 3 months of observation. Lumen is well preserved. Development of all the components of the wall of the fallopian tube is well demarcated (L = lumen, E = epithelium, M = muscle layer, S = serosal layer). (b) Normal fallopian tube. 10x10 magnification (compare with Fig. 3a). (c) Mucosal and smooth muscle layer. 10x25 magnification. (d) Mucosal cells with cilia. 10x40 magnification, arrow indicates cilia bearing cells (see text). (e) Masson's Tri-CHrome stain. (10x10 magnification) Smooth muscles (M), in brick red colour and Connective tissue (C), in green colour. (f) Regeneration beyond the line of anastomosis (H & E Stain 10x10 magnification). Arrow "A" shows line of anastomosis, held between the two sutures S i and S ii. Components of the wall of the fallopian tube are: Lumen (L), Epithelium (E), Smooth Muscle layer (M), and Serosal layer (S).
Fig. 4—Embryology of development of fallopian tube and uterus. (a) Germ Disk: Intra embryonic mesoderm showing ectoderm (E), endoderm (e) and parts of the mesoderm (M): A. paraxial mesoderm, B. intermediate cell mass mesoderm & C. lateral plate mesoderm with coelomic cavity. (b) Transverse section of the 5 mm embryo showing intermediate cell mass mesoderm—the nephrogenic cord covered with peritoneum which is the lining of the intra embryonic coelome. The dark line is the covering epithelium of the coelome—the coelomic epithelium which is nothing but peritoneum. (c) Nephrogenic cord structures showing different structures of the nephrogenic cord. The dark line is the covering epithelium of the coelome—the coelomic epithelium invaginates into the surrounding mesenchyme and forms the paramesonephric duct. The coelomic epithelium is peritoneum of the abdominal cavity. Therefore in fact peritoneum cells are responsible for the formation of the fallopian tube. (d) Formation of paramesonephric duct. (e) Formation of fallopian tube (stage I) and (f) utero-vaginal canal (stage II): The paramesonephric duct form fallopian tube one on each side of the midline. The two tubes unite lower down in mid line to form the uterus and uterovaginal canal.
The regeneration capacity is rare minimum or even absent. In nature the regeneration exists in plants and lower animals. A plant can be grown into full tree from a small cutting of the stem of the plant, if the small cutting is put in a proper soil, environment and proper water, moisture in the soil. Otherwise the cutting will dry off and die. Similarly in lower animals, the eye lens can be grown by newts if amputated, tail of the lizard can be grown if severed, the claws of the crabs and lobsters can be re-grown if injured and lost.

The regeneration exists in nature. Therefor regeneration is a possibility in higher forms of life and the man as well. The pluripotent stem cells do exist in tissues of higher forms of fully developed animals, the mammals and the man. These cells are responsible for repair, healing and replacement of lost cells of tissues and organs of the body. Also these cells can undergo metaplastic transformation on new pathways and form a totally new different tissue if exposed to irritation or abnormal conditions. For example, in chronic peritonitis cases the peritoneal stem cells show formation of squamous nests, papillary projections, smooth muscle formation, cartilagenous changes and even osseous tissue formation. This is confirmed by the pathologists as well. This new tissue formation is limited to tissues which are formed from mesodermal germ layer of the developing embryo, since peritoneum stem cells are developed from germ layer mesoderm only. By exploiting these facts of embryology, metaplastic transformation capacity of stem cells of the peritoneum to form mesodermal tissues, when environment is altered, the regeneration of ureter was successfully achieved in dogs and monkeys. The regeneration of abdominal wall aponoeurosis from stem cells of the peritoneum was achieved and utilized in the management of difficult cases of primary and recurrent incisional hernias. Based on similar principles the human ailments of complex genito urinary rectal fistulae were managed by using peritoneum for urethroplasty and as an interpositional tissue.

The metaplasia of fully differentiated tissues cells is often considered as abnormal and pathological. If the stem cells in the process of differentiation and proliferation to replace morphologically different tissues, are subjected to undergo a change which is desired in the new situation and environment the desired metaplasia is a possibility and may be responsible for regeneration of tissues and organs. Accordingly the regeneration of fallopian tube is attempted in this study.

Even though the scope of this experimental study is limited to test the hypothesis and study the extent to which the components of the wall of the fallopian tube can be grown, the applied aspect of the results can be far reaching. Various tissues used in tubal plastic surgery are verniform appendix, human umbilical vein, vein and artery implants, amnion, ileum, seromuscular ileal grafts, with various success rates. The reason for failure could be that these different tissue substitutes were not integrated in the process of natural wound healing and tissue repair. The biodegradable synthetic microporous artificial vascular grafts show excellent healing characteristics. Despite notable improvement in over all results of tuboplasty a suitable method is still needed for replacement of part or whole of the fallopian tube to correct the extensive damage.

The fallopian tube and peritoneum, both are developed from germ layer mesoderm—the intermediate cell mass mesoderm and lateral plate mesoderm, respectively (Fig. 4a). The urogenital cord with its component structures is covered by the epithelial lining of the coelomic cavity of lateral plate mesoderm which becomes the peritoneum, pleura and pericardium of the body cavity (Fig. 4b). The fallopian tube is formed from the paramesonephric duct of the urogenital cord of the urogenital ridge. The paramesonephric duct is formed by the invagination of the coelomic epithelium into the surrounding mesenchyme (Fig. 4c). Therefore it can be inferred that the fallopian tube is developed from the cells of the peritoneum. But the regional and functional needs of the local tissue system were different, the environment to which these cells migrated in embryo were different and therefore remained different tissue wise and functioned differently. Otherwise these peritoneal pluripotent cells had the capacity to form the fallopian tube. Therefore the peritoneum is used in the present experiment.

The fate of the germ layer cells in embryo depends up on intrinsic and extrinsic cell factors, cell
movements and location to which these cells are exposed in embryo along with remote control mechanism of surrounding and distant tissues. The other factor being the time needed for the development, coupled with functional need of the location to which these cells migrate. In fact it is well known that the vertebrate development is due to environmental factors rather than the genetic coding. Therefore an attempt has been made to study the effect of exposure of peritoneal stem cells, to a different location of fallopian tube tissue system, in this experiment.

The peritoneum has single layer of stem cells on a tough basement membrane. These stem cells maintain pluripotent membrane and are capable of undergoing metaplastic transformation to tissues of mesodermal origin as explained above. The fallopian tube is developed from germ layer mesoderm. Therefore this scientific fact has been exploited in this experiment.

The surgical technique provides the needed cell movement to the new location of fallopian tube tissue system. The inherent intrinsic cell coding and creative intelligence of stem cells of the peritoneum to differentiate and proliferate on a new pathway coupled with forced new functional need of the changed environment triggered the desired events. Probably these factors are responsible for inducing desired changes to regenerate components of the wall of the fallopian tube.

References of specific direct studies regarding healing of fallopian tube and the possibility of migration of cells from proximal and distal cut ends of the fallopian tube are not available. However the other experimental studies on different tubular organs like ureter, oesophagus, duodenum, vein pelvis and pelviureteric junction, etc. reveal that the healing and tissue formation is possible by migration of cells to a few millimeters only from the cut ends but lead to severe stricture formation and contracture at the severed site. The entire tissue or organ formation has not been observed in the grafted areas in any of these studies. Some of these studies have even used peritoneum from adjacent peritoneal envelope or peritoneum covered with fascia and transverse abdominis muscle. The migration of cells from cut ends in these experiments could have generated tissues of entire organ. Apart from this the experimental failure in regenerating tissues using different materials and peritoneum not from contiguous embryonal segment region (Matapurkar, unpublished data) also suggests that the cell migration is not responsible for entire organ regeneration. The migrated cells have no capacity to survive till properly implanted in the area and resulted in undue scarring responsible for stricture formation. On the contrary the stem cells of the peritoneum have unique property to differentiate and proliferate and even the free peritoneal grafts survive. Not only this, even the free-floating peritoneal cells also have the capacity to differentiate and proliferate to help in the healing. By this property the peritoneum acquires tremendous speed in healing.

The chronic inflammatory cell infiltration seen in the mucosal and serosal layers is probably the result of the local inflammatory response of the macrophages present in the peritoneum. The microsurgical reconstruction of the fallopian tube studied by Guawesky et al. showed regeneration of mucosa. The ciliogenesis after salpingostomy in rabbit has been observed between 2nd and 3rd week. These studies were in artificially created hydrosalpinx by tube occlusion. The use of biodegradable synthetic micro porous tube as uterine horn graft has been attempted for healing and regeneration of fallopian tube and uterus in rats. These produce obstruction at the junction of fallopian tube and uterus. There is no mention of cilia/mucosal regeneration. The use of splints contributing to the tubal surgery by biodegradable microporous artificial graft has been suggested. Absence of cilia in majority of present experiments was due to probably that there was a long time gap between fixation and actual sectioning of the biopsy tissue which increased the friability of the tissue resulting in shedding up of the cilia. However in one dog where oestrogen was used, parentally two weeks prior to obtaining the biopsy tissue, the cilia were observed in the region of the grafted tube. In fact the tube part which was excised for regeneration was the isthmus area which has very scanty cilia bearing cells in the mucosa. Moreover the population of cilia is dependent upon the phase of the oestrous cycle which was also not taken into consideration in this experiment.

The metaplastic transformation capacity of stem cells of the peritoneum has been exploited. The regeneration of the components of the wall of the fallopian tube is possible by desired metaplasia of the stem cells of the peritoneum in the region of the fallopian tube. The scientific rationale of the hypothesis and the factors responsible for
regeneration of the fallopian tube are discussed. However the authentication requires specifically targeted studies regarding the extrinsic and intrinsic factors responsible for regeneration of the fallopian tube.

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References