Locating an SPLN has been a challenging task since the era of open thoracotomies. It is even more difficult in a thoracoscopic situation because finger palpation is far less effective. As a consequence, many techniques to assist in the localization of SPLNs have been innovated, with these techniques broadly belonging to 1 of 2 completely different strategies: intraoperative techniques (eg, finger palpation and ultrasound) and preoperative placement of a marker (eg, hook-wire, dye, contrast medium, and radiotracer).[4]

Intraoperative techniques have the virtue of noninvasiveness, and to our knowledge no major complications as a result of these techniques have been reported. Even though intraoperative US is more effective than finger palpation, with a success rate of 70% to 100 % in selected patients,[4] this technique has several limitations.[5] Successful localization using intraoperative US is highly dependent on operator skill. Also, localization is difficult in cases of emphysematous change or incomplete collapse of the target lung. Success rates are further reduced in cases of small nodules less than 1 cm in size or deep lesions more than 1 cm from the visceral pleura. Soft nodular ground-glass opacities observed in bronchoalveolar cell carcinomas are also difficult to localize using US. Because of these limitations, intraoperative US has little versatility as a localization technique.

The use of markers is a viable option for surgeons to overcome the limitations of intraoperative localization techniques. The implantation or delivery procedures of several kinds of markers can be classified into 2 categories according to their approaches: a percutaneous pneumocentesis approach and a transbronchial approach with a bronchoscope. Generally, percutaneous implantation of a marker is highly associated with complications such as pneumothorax (8%–50%), pulmonary hemorrhage (12%–35%), and pleuritic pain (5%–6%).[4] In addition, several authors have raised caution on the risk of fatal air embolisms induced by hook-wire implantation.[18] In contrast, the transbronchial approach can theoretically eliminate the risk of complications related to the percutaneous approach. In our experiments, no major complications were observed related to bronchoscopic delivery of RFID tags, and the time required for marking ranged from 5 to 34 minutes (median, 11 minutes). Bronchoscopic procedures can be a distressing experience for patients, but the transbronchial approach is less invasive than the percutaneous approach.

However, some markers have been used despite their negative aspects. Time constraints are the main cause of failure in the case of dye or radiotracer marking, as the diffusion of dyes and the decay of radiotracers prevent surgeons from successful localization of SPLNs. These shortcomings also lead to difficulties in scheduling preoperative procedures and surgical resection. It has been recommended that surgery be performed within 24 hours after injection of dye and within 12 hours after radiotracer administration.[4,5] The RFID marking system is free from these time constraints and can provide flexibility in the time interval between the marking procedure and surgery.

In the context of intraoperative fluoroscopy, a contrast medium (ie, barium or lipiodol) is consistently reported as the most effective and reliable marker: Watanabe and colleagues[19] reported a success rate of 100% from their experience of 174 nodules; Asano and associates[20] emphasized a success rate of 100% for 31 pure ground-glass nodules less than 10 mm in size; and Okumura and coworkers[21] stated that good surgical margins could be assured with this technique and that multiple markings can be achieved by transbronchial injection of barium with minimum invasiveness. Unfortunately, despite these merits, the radiation exposure for operators and patients cannot be overlooked. Unnecessary exposure to medical radiation should be avoided if possible, and the use of RFID technology presents an alternative for the purpose of SPLN localization.

The RFID marking system has several positive aspects. The first merit is that of quick detection with simple operability. The system requires only a locating probe in the surgical field. During thoracoscopic surgical procedures, localization of the RFID tag was promptly achieved without the need for unwieldy instruments such as a fluoroscope. As shown in our experiments, the time required for tag detection after insertion of the locating probe ranged from 10 to 105 seconds (median, 27 seconds). Quick detection of the tag and swift localization of the target lesion were achieved by simply listening and reacting to the audio cues from the system; there was no need for operators to shift their gaze from the surgical field or the monitor for the thoracoscope. The simple operability of the system also exempts thoracic surgeons from having to become skilled in an unfamiliar technique such as US interpretation or from the threat of radiation exposure. The second and more important merit is that the 1-mm small wireless marker enables the pinpoint localization of target lesions; the system provides millimeter-level ranging capability to enhance localization accuracy. As a consequence, an appropriate distance from the lesion to the resection line is ensured because the operator can confirm that the RFID marker is included within the lung parenchyma to be resected. In our study with pseudolesions, surgical margins of resected specimens ranged from 8 to 12 mm (median, 11 mm). Accurate localization enables thoracic surgeons to perform sublobar resections with reduced risk of local recurrence in a short procedure time. Third, the labeling of multiple lesions or multiple marking of a single lesion can be achieved in this system because each tag has its own unique identifiable number.

This study has the following limitations. Our prototype system has an effective communication range of 7 mm, mainly owing to limitations in the power supply from the locating probe. Although 7 mm is adequate for animal experiments, an effective range of 30 mm may be required

for clinical use in human lungs. Improvements to the effective range are in progress and can be achieved with further customization of the tag and the probe.

The second limitation is that dislocation of the implanted tag is a problem of critical importance for the RFID marking system. The reason for the sole detection failure in our experiments was the dislocation of the tag to the central airway. To address this problem, we are developing a tag anchoring function. For the purpose of lung tumor tracking during radiation therapy, the use of an RFID tag with anchoring legs was reported to have an 87% successful stabilization rate for a period of 60 days.[22] In addition to the anchoring function, the coating of RFID tags is another modification under consideration owing to the possibility of accidental ingestion of a dislocated tag into the digestive tract.

The third limitation is related to the safety of the system. We did not encounter any instances of adverse effects caused by the tag, but the study period was limited to 3 days at its longest. The safety of the in vivo use of RFID tags has been endorsed by long-term experiments in a canine subcutaneous implantation model,[23] as well as by routine use in livestock farming. Although we have had limited experience with RFID tag placement into a small airway, Mayse and coworkers[16] have reported no complications in similar experimental implantation of 60 days. To our knowledge, no other published report has described the long-term implantation effects of RFID tags into airways. Further investigation is therefore required into the safety of this system.

The additional cost, time, and radiation exposure could be a demerit of this new method. However, the preoperative marking procedure under CT guidance may be appropriate for cases in which the target is a small peripheral lesion that is difficult to locate under fluoroscopy. RFID tags in commercial mass production are expected to be similar in cost to conventional markers including hook-wires or dye. In general, we estimate that the additional cost, time, and radiation exposure for the RFID marking method are expected to be in the same range as conventional methods that require CT guidance. A cost-benefit analysis is required to determine the best role and use of such techniques in the management of small peripheral lung nodules.

## CONCLUSIONS

The feasibility of our RFID marking system for SPLN localization was demonstrated in a canine model. Further developmental work is underway to improve the effective range, to equip the tags with an anchoring function, and to confirm the safety in implantation.

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# Development of a composite and vascularized tracheal scaffold in the omentum for *in situ* tissue engineering: a canine model

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## Abstract

**OBJECTIVES**: We herein report on development of a composite (synthetic and biological) tracheal scaffold with vascularized autologous connective tissue in the omentum, followed by *in situ* tissue engineering of the composite scaffold with the pedicled omentum. In this preliminary report, we focus on development and evaluation of the vascularized autologous connective tissue in the omentum.

**METHODS**: In animal experiment 1, a polypropylene framework as a synthetic component was placed in the omental sac for 3 weeks and another was placed in the pouch of Douglas as a control in five beagle dogs. In animal experiment 2, a polypropylene framework placed in the omental sac for 3 weeks was compared with a polypropylene framework coated with porcine atelocollagen, which was also placed in the omental sac in another five dogs, to investigate whether the coating of porcine atelocollagen contributes to development of more vascularized connective tissue. Macroscopic, radiological and histological evaluations were performed for developed autologous connective tissue on the frameworks, with a focus on its thickness and capillary vessels.

**RESULTS**: In animal experiment 1, the polypropylene framework in the omentum developed a composite tracheal scaffold with homogeneous and significantly thicker (2.6 ± 0.5 vs 1.2 ± 0.4 mm, $P < 0.0001$) connective tissue in which more capillary vessels per 10-power field of view (3.5 ± 2.2 vs 0 ± 0, $P = 0.015$) were identified, compared with the control in the pouch of Douglas. In animal experiment 2, the omentum developed significantly thicker connective tissue on the polypropylene framework coated with porcine atelocollagen (3.6 ± 0.7 vs 2.2 ± 0.4 mm, $P < 0.0001$) in which not significantly more capillary vessels were identified (3.5 ± 2.2 vs 5.0 ± 2.7, $P = 0.12$), compared with the framework that was not coated.

**CONCLUSIONS**: Placement of the polypropylene framework in the omental sac resulted in development of homogeneous and vascularized autologous connective tissue on the polypropylene framework for a composite tracheal scaffold. The framework coated with porcine atelocollagen did not show an additional benefit in inducing vascularization. This preliminary report will be followed by the long-term evaluations of *in situ* tissue engineering of the composite tracheal scaffold.

**Keywords:** Trachea • Omentum • Prosthesis

## INTRODUCTION

A variety of artificial tracheas have been designed and assessed for inter-position, but so far none has proved satisfactory for clinical use [1]. The first human case for a circumferential airway replacement was performed by Macchiarini *et al.* in 2008 using a decellularized human tracheal allograft that was cellularized with epithelial cells and mesenchymal stem cell-derived chondrocytes by *in vitro* tissue engineering [2]. However, a non-circumferential (only a cartilaginous portion of a trachea) replacement for invading thyroid malignancy was performed by Omori *et al.* in 2002, using a polypropylene mesh framework coated with porcine atelocollagen and *in situ* tissue engineering [3], which was originally developed in our laboratory.

For a successful airway replacement, a scaffold, which can be a biological (allograft/homograft), synthetic or composite [4], requires epithelial cellularization by *in vitro* [2, 5], *in situ* [6] or heterotropic [7] tissue engineering, which can be promoted by bioactive molecules [8].

We developed an airway scaffold that is a polypropylene framework (as a synthetic component) coated with porcine atelocollagen (as a biological component) for *in situ* tissue engineering in tracheobronchial replacements [9, 10]. Our previous attempts to replace circumferentially the airway with this type of prosthesis in

canine models revealed that its durability extends past 5 years, whereas long-term observations showed incomplete epithelialization on the prosthesis and anastomotic stenosis [9, 10]; similar findings were also noted in the first human circumferential airway replacement in the follow-up [11].

To improve incomplete epithelialization and anastomotic stenosis, we applied a pedicled omentum in tracheobronchial reconstruction procedures; however, the omental transposition procedure alone has not been sufficient to resolve incomplete epithelialization and anastomotic stenosis [9, 10, 12].

In this study, we report and evaluate the development of a composite (synthetic and biological) tracheal prosthesis with vascularized autologous connective tissue developed by the ome-ntum. *In situ* tissue engineering of the composite scaffold with the pedicled omentum (Fig. 1) will be evaluated and described in our next report.

## MATERIALS AND METHODS

### Polypropylene framework for a tracheal scaffold

A polypropylene mesh framework was made using a method described in a previous report [10]. The framework is a 0.8-mm-thick polypropylene mesh (Marlex mesh; CR Bard, Inc., Billerica, MA, USA) cylinder that is 25 mm long and has an internal diameter of 20 mm, which is reinforced with four rings of polypropylene monofilament string (1 mm in diameter). This cylindrical mesh framework was exposed to a corona discharge at 9 kV for 5 min to render its surface hydrophilic (Fig. 2A); this was used in animal experiment 1. In animal experiment 2, the polypropylene framework was coated with a 5-mm-thick porcine atelocollagen layer with a pore size ranging from 100 to 500 × $10^{-6}$ m (Fig. 2B).

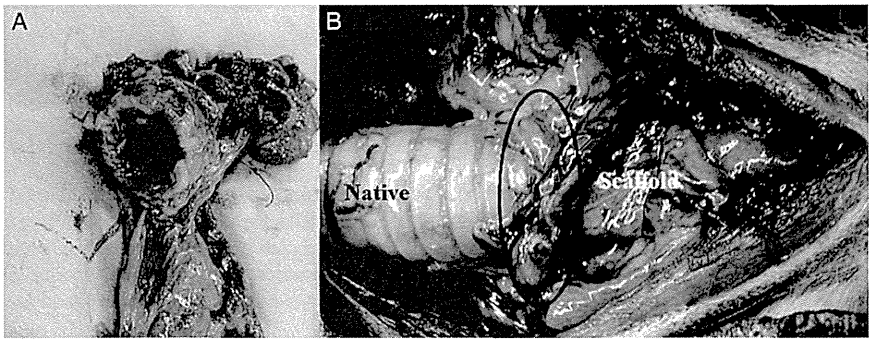


**Figure 1:** *In situ* tissue engineering of a composite tracheal scaffold, placed with a pedicled omentum through a substernal route. (**A**) Pedicled omental flap containing a composite and vascularized tracheal scaffold, (**B**) anastomosed with a native trachea (the proximal anastomosis is circled).

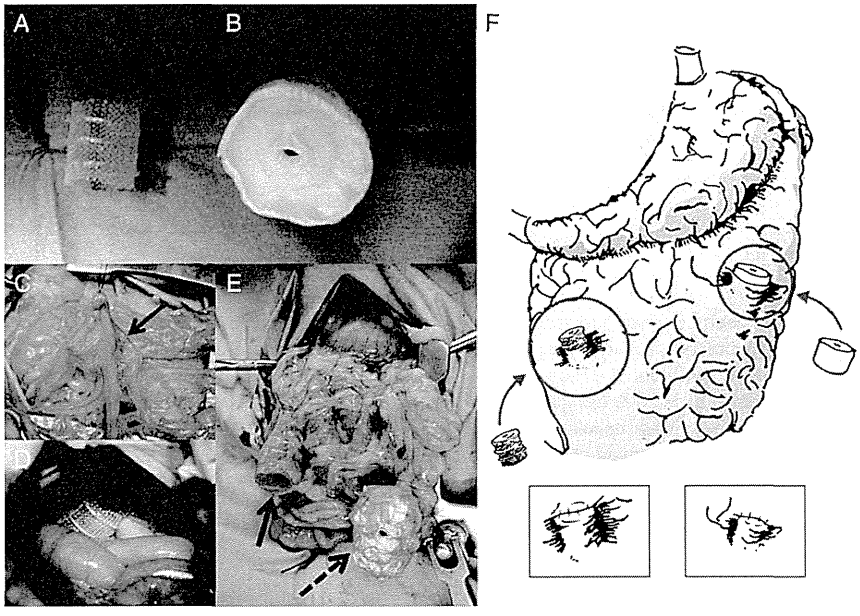


**Figure 2:** The polypropylene frameworks and their intra-abdominal placements. (**A**) A polypropylene framework prior to placement; (**B**) a polypropylene framework coated with porcine atelocollagen prior to placement; (**C**) a polypropylene framework (indicated by an arrow) placed in the omentum in animal experiment 1; (**D**) a polypropylene framework placed in the pouch of Douglas as a control in animal experiment 1; (**E**) a polypropylene framework (a solid arrow) and a polypropylene framework coated with porcine atelocollagen (a dotted arrow) placed in the omentum in animal experiment 2; (**F**) an illustration of animal experiment 2.

The porcine atelocollagen layer was developed as follows [10]: the polypropylene framework was placed in a Teflon mould. Then, 1% collagen solution (Nippon Meat Packers, Inc., Osaka, Japan) was stirred at 8000 rpm for 15 min, and then poured into the space between the outer mould and the inner tube, and then freeze-dried. During this freeze-drying process, the cast collagen became a porous structure with a pore size range of 100 to $500 \times 10^{-6}$ m. A 5-mm-thick collagen layer was formed on both internal and external luminal surfaces. Finally, the prosthesis was heated at 140°C under vacuum pressure for a 24-h dehydrothermal treatment session to induce moderate cross-linkage of the collagen molecules.

## Animal experiments

***Animal experiment 1.*** Development of a composite scaffold from polypropylene frameworks (in the omentum versus in the pouch of Douglas).

Five adult beagle dogs, weighing 8–14 kg, were anaesthetized with an intramuscular administration of 15 mg/kg ketamine hydrochloride and 7 mg/kg xylazine and then intubated with an endotracheal tube. Mechanical ventilation was maintained using inhalational sevoflurane. A 500-mg dose of ampicillin was injected intramuscularly prior to the skin incision. In each dog, a polypropylene framework was placed in the omental sac after making a slit on the omental peritoneum via an upper mid-line laparotomy (Fig. 2C), and another polypropylene framework was placed in the pouch of Douglas as a control (Fig. 2D). Both were extracted 3 weeks afterwards via a reoperative laparotomy. After sectioning each specimen into two transversely, the thickness of the developed connective tissue was measured with a ruler at three different points (every 120°) on the circumference.

***Animal experiment 2.*** Development of a composite scaffold in the omentum (from polypropylene frameworks versus from polypropylene frameworks coated with a porcine atelocollagen layer).

Another five adult beagle dogs, weighing 8–14 kg, were anaesthesized in the same way as above. A 500-mg dose of ampicillin was injected intramuscularly prior to the skin incision. Two types of framework, as mentioned above, were both placed in the omental sac (Fig. 2E and F). Both were extracted 3 weeks afterwards as in Animal experiment 1, and the thickness of the developed connective tissue was measured in the same way.

All surgical procedures were performed by board-certified surgeons (Masatsugu Hamaji and Fumitsugu Kojima) in accordance with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH Public-ation No. 85-23, revised 1985). The experimental protocol was approved by the Animal Experimental Committee of Kyoto University.

Following first laparotomies (for placement of polypropylene frameworks) or second laparotomies (for extraction of the frameworks), all the dogs received the same regular care as preoperatively. Macroscopic findings at reoperative laparotomies were obtained with a focus on developed connective tissue.

## Radiological analysis of developing composite scaffolds

To follow-up the process of developing composite scaffolds in the omentum, four dogs were randomly selected from animal experiment 2 for abdominal computed tomography (CT). All CT images from the diaphragm to the hip joints were obtained with a 16-row multidetector CT scanner (Alexion 16, Toshiba Medical systems, Tochigi, Japan) with an intramuscular administration of ketamine and xylazine on postoperative day (POD) 0, 7, 14 and 21. The images were obtained in the helical mode with 120 kV voltage, 50 mA per section, a $512 \times 512$ matrix and 7-mm slice thickness. All images were interpreted by a board-certified radiologist (Sho Koyasu).

## Histological analysis of developed connective tissue on the frameworks

In animal experiment 1, two scaffolds developed from polypropylene frameworks in the pouch of Doulgas and two scaffolds developed from polypropylene frameworks in the omentum (from two dogs) were sent for histological analysis by light microscopy after staining with haematoxylin and eosin (H&E), Masson trichrome (MT) and alpha-smooth muscle actin (α-SMA). The number of capillary vessels was counted in three randomly selected 10-power fields of view after staining with H&E and compared between the two kinds of scaffold. In animal experiment 2, two scaffolds developed from polypropylene frameworks in the omentum and two scaffolds developed from polypropylene frameworks coated with porcine atelocollagen in the omentum were analysed in the same way. The number of capillary vessels was counted in three randomly selected 10-power fields of view and compared between the two kinds of scaffold. All specimens were examined by a board-certified pathologist (T.T.).

## Statistical analysis

Descriptive statistics for continuous variables are reported as mean ± standard deviation. For the comparison of continuous variables, the Mann–Whitney *U*-test was used as appropriate. All statistical tests were two-sided, and a *P*-value <0.05 was defined as statistically significant. JMP version 10.0.1 software (SAS Institute, Cary, NC, USA) was used for all analyses.

# RESULTS

## Clinical courses and macroscopic findings of developed composite scaffolds

All dogs uneventfully survived the sequential laparotomies in animal experiments 1 and 2.

In animal experiment 1, on reoperative laparotomies, there were minimal to mild adhesions between the abdominal wall and the omentum without peritoneal fluid. No migration was noted in frameworks placed in the omentum, whereas two (40%) polypropylene frameworks placed in the pouch of Douglas were noted to have cephalad migrations; the others involved a portion of the small bowels in the lumen (Fig. 3A, in animal experiment 1). The omentum was found to be focally hyperplastic around the framework. Regarding scaffolds developed from polypropylene frameworks in the pouch of Douglas, only thin layers (1.2 ± 0.4 mm) of connective tissue developed on the frameworks (Fig. 3B), with a minimal area of the mesh exposed, whereas homogeneously thick

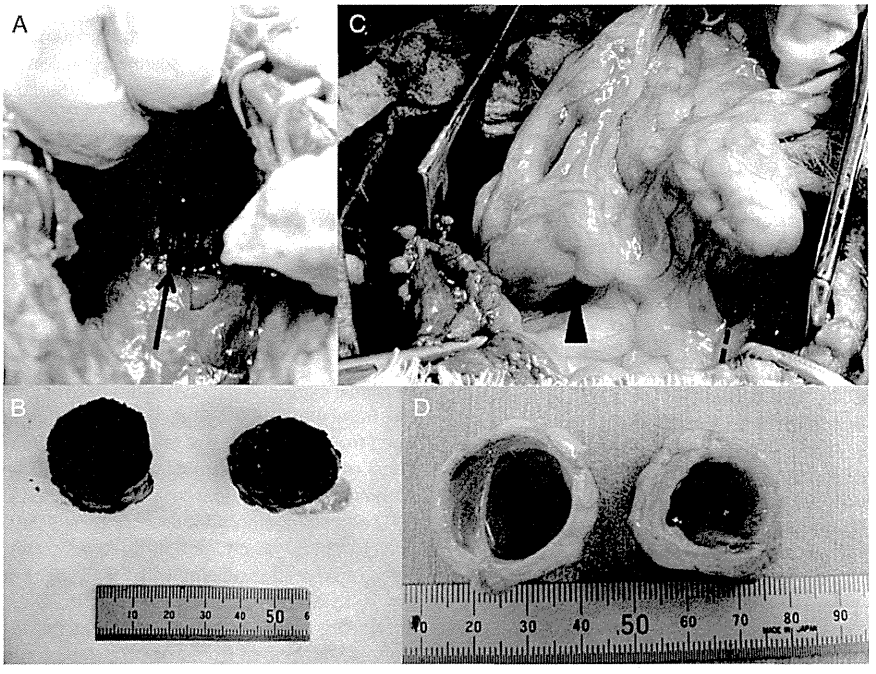


**Figure 3:** The developed scaffolds at reoperative laparotomies (3 weeks after placement). (**A**) The small bowels adhered to the lumen of the polypropylene framework (solid arrow) in animal experiment 1. (**B**) A scaffold developed from polypropylene framework in the pouch of Douglas (left) and in the omentum (right). (**C**) Polypropylene framework (arrowhead) and the polypropylene framework coated with porcine atelocollagen (dotted arrow) contained in the omentum in animal experiment 2. (**D**) A scaffold developed in the omentum from a polypropylene framework (left) and a polypropylene framework coated with porcine atelocollagen (right).

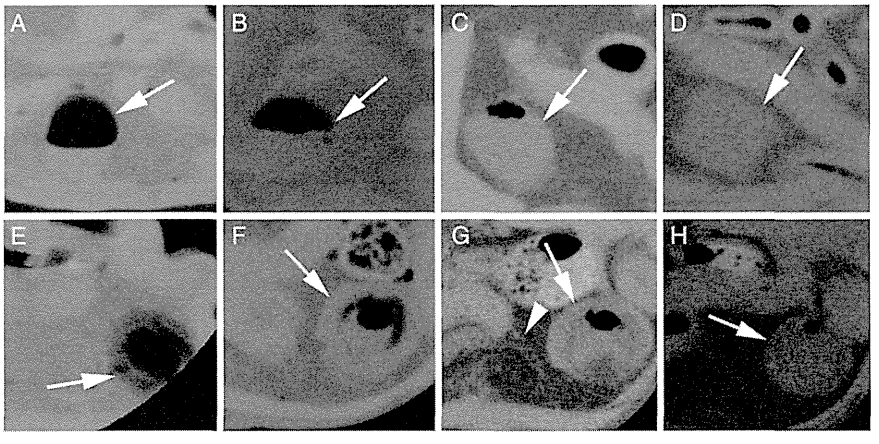


**Figure 4:** Abdominal computed tomography (CT) images of the polypropylene frameworks on postoperative day (POD) 0 (**A**), 7 (**B**), 14 (**C**) and 21 (**D**) and the polypropylene framework coated with porcine atelocollagen on POD 0 (**E**), 7 (**F**), 14 (**G**) and 21 (**H**) in animal experiment 2. Arrows in images show frameworks. The polypropylene framework was recognized as containing fluid that increased in volume over time (**A**–**D**). In the polypropylene framework coated with porcine atelocollagen, a layer that was shown as having mixed air and soft tissue density gradually decreased and disappeared on POD 14 (**G**), and soft tissue density growing on the framework was observed (**F**–**H**).

layers (2.6 ± 0.5 mm) developed on the frameworks in the omentum without any area exposed (Fig. 3C). Serous fluid was contained in the framework in the omentum. There was a statistically significant difference in the thickness of the connective tissue between scaffolds developed in the omentum and those in the pouch of Douglas (2.6 ± 0.5 and 1.2 ± 0.4 mm, $P$ <0.0001).

In animal experiment 2, the omentum was found to be focally hyperplastic around the frameworks (Fig. 3C). Regarding the polypropylene frameworks or polypropylene frameworks with conjugated porcine atelocollagen from the omentum, connective tissue developed on the frameworks without any mesh exposed (Fig. 3D). Both of the developed scaffolds appeared to be waterproof and airtight and contained serous fluid. There was a statistically significant difference in the thickness of developed connective tissue between polypropylene frameworks uncoated and those with with porcine atelocollagen (2.2 ± 0.4 and 3.6 ± 0.7 mm, respectively, $P$ <0.0001).

## Radiological findings of developing composite scaffolds in the omentum on abdominal computed tomography

Representative images are shown in Fig. 4. The polypropylene framework in the omentum was recognized as containing fluid that increased in volume over time (Fig. 4A–D). The polypropylene frameworks coated with porcine atelocollagen in the omentum are shown in Fig. 4E–H, and the coating porcine atelocollagen on the framework is recognized as a layer of air mixed with soft tissue density on the lumen on POD 0 (Fig. 4E, arrow). This layer was replaced gradually with soft tissue density (Fig. 4F–H).

## Histological findings of the connective tissue developed on the frameworks

In animal experiment 1, the number of capillary vessels in a 10-power field of view was 0 ± 0 in connective tissue of scaffolds developed in the pouch of Douglas and 4.5 ± 3.0 in the connective tissue of ones developed in the omentum, with a statistically significant difference ($P = 0.015$).

In animal experiment 2, the number of capillary vessels in a 10-power field of view was 3.5 ± 2.2 in the connective tissue of scaffolds developed from polypropylene frameworks in the omentum versus 5.0 ± 2.7 in the connective tissue of scaffolds developed from polypropylene frameworks coated with porcine atelocollagen in the omentum, but the difference was not statistically significant ($P = 0.15$).

The connective tissue developed on the polypropylene frameworks in the omentum in animal experiment 1 showed almost the same histological findings as that on the frameworks in the omentum in animal experiment 2. Therefore, images from animal experiment 2 are shown.

Shown in Fig. 5 are microscopic (original magnification ×10) examinations. In Fig. 5D and G, fibromuscular fibres developed and surrounded the polypropylene mesh (M) with irregular capillary vessels (arrows in magnified parts). All sections with MT staining suggested that the connective tissue was rich in collagenous fibres (Fig. 5B, E and H), produced by fibroblasts, shown with α-SMA staining (Fig. 5C, F and I).

## DISCUSSION

Non-circumferential (cartilaginous portion only) replacement of a human airway with a prosthesis has already been successfully performed after resection of a cartilaginous portion of cervical trachea in 2002 [3], whereas a circumferential replacement of human airway is more challenging and was first performed by Macchiarini *et al.* in 2008 with a decellularized human tracheal graft that was cellularized by *in vitro* tissue engineering [2].

An airway prosthesis comprises a scaffold that is biological, synthetic or composite (biological and synthetic) and has tissue-engineered epithelial cells on it. It is still controversial which type

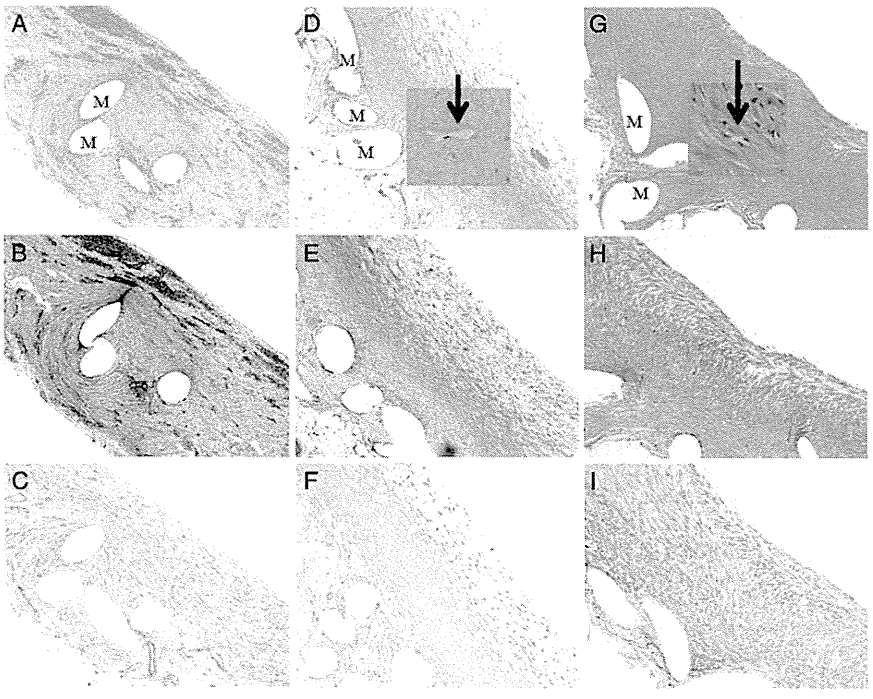


**Figure 5:** Sections of connective tissue of a scaffold developed from a polypropylene framework in the pouch of Douglas in animal experiment 1: (**A**) H&E staining, original magnification ×10; (**B**) MT staining, original magnification ×10; and (**C**) α-SMA staining, original magnification ×10. Sections of connective tissue of a scaffold developed from a polypropylene framework in the omentum in animal experiment 2: (**D**) H&E staining, ×10; (**E**) MT staining, ×10; and (**F**) α-SMA staining, ×10. Sections of a scaffold developed from a polypropylene framework coated with porcine atelocollagen in the omentum in animal experiment 2: (**G**) H&E staining, ×10; (**H**) MT staining, ×10; and (**I**) α-SMA staining, ×10). Fibromuscular fibres developed and surrounded the polypropylene mesh and spiral with irregular capillary vessels (indicated by an arrow in the magnified fields of **D** and **G**). In addition, lymphocytes infiltrated the connective tissue of all sections, suggesting chronic inflammatory processes. The connective tissue of all scaffolds demonstrated α-SM fibres or α-SMA (+) spindle cells with a connective tissue matrix (**C**, **F**, **I**).

of scaffold and which type of engineering are most suitable for developing an airway prosthesis. Regarding the scaffold, the advantages of a biological one included its biocompatibility and its excellent environment for cellularization by tissue engineering, whereas a synthetic scaffold has the advantages of no dependency on donors and easy handling. As for tissue engineering (prior to grafting), *in vitro* or heterotropic, it is expensive and requires technology for cell culture and implantation, whereas *in situ* tissue engineering (following grafting) has no cells on the scaffold at first and, therefore, depends on the anastomosis as a sole source for epithelial cellularization.

Airway stenosis near the anastomoses is one major problem regarding an airway prosthesis in either humans [11] or a canine model [6, 9, 10]. Macchiarini *et al.* experienced one (11.1%) with a stenosis out of nine patients in the long-term follow-up [4], while awaiting long-term (>1 year) follow-up of heterotropic tissue engineering [7]. In a canine model, the long-term incidence of anastomotic stenosis ranged from 25 to 38% without omental transposition. To resolve anastomotic stenosis and incomplete epithelialization, the omentum was previously applied in our laboratory and by other groups to wrap the anastomosis of a native airway and a prosthesis by taking advantage of its high vasculature [11–13]. The omental transposition procedure at airway grafting lowered somewhat the incidence of an anastomotic stenosis [9], but did not resolve it completely [9, 10, 12].

The omentum is known to not only vascularize other tissues, but also modulate inflammatory reactions, through which it produces connective tissue either in acute inflammatory reactions or in the subacute wound healing process [14]. Our findings in animal experiment 1 confirmed that the functions of the omentum can be applied to developing a composite scaffold for *in situ* tissue engineering, because vascularization in the scaffold is a key to successful epithelial cellularization on the scaffold [15]. Owing to its rich vasculature, the omentum appears to be superior to muscles, which are potential alternatives, in developing a composite and vascularized tracheal scaffold.

In this preliminary study, an attempt was made to develop a novel method for developing vascularized connective tissue on a synthetic framework, which could provide an excellent environment as a composite scaffold for *in situ* tissue engineering following grafting. In animal experiment 1, the control that was placed in the pouch of Douglas showed no capillary vessels in the specimen sections or an unreliable thickness (1.2 ± 0.4 mm) given that the thickness of the polypropylene mesh was 0.8 mm. In Animal experiment 2, porcine atelocollagen coating the polypropylene framework failed to show an additional benefit in vascularizing the polypropylene framework. Although the polypropylene framework coated with porcine atelocollagen in the omentum did develop thicker autologous connective tissue (3.6 ± 0.7 mm) on the scaffold, the thickness appears more than required, given that the thickness of a normal trachea is estimated to be 1–3 mm on CT [16]. Moreover, too thick connective tissue might be a barrier to neck movements. These findings suggest that porcine atelocollagen coating the polypropylene framework does not have additional benefits in developing a composite and vascularized scaffold.

The serial CT images on the polypropylene scaffold successfully identified and followed up the process of developing autologous connective tissue, which apparently plateaued between 14 and 21 days from placement. The radiological findings suggested that the development process can be tracked to some degree but revealed limitations in estimating the thickness.

The limitations of the study included use of canine models as a substitute for human subjects. In addition, the omentum can be unreliable in cases with a history of prior laparotomy and/or an upper abdominal procedure. Prior to the second stage (tracheal grafting), 3 weeks are required for connective tissue development, which is not ideal for emergent or semiemergent treatments.

Our results suggest that development of a composite tracheal scaffold with vascularized autologous connective tissue is feasible. We need to evaluate carefully the long-term outcomes of *in situ* tissue engineering in further studies, but this composite tracheal scaffold could be a reasonable alternative to our previous scaffolds or to those developed by other groups. Our next report will focus on long-term outcomes of *in situ* tissue engineering of the composite scaffolds following grafting.

**Conflict of interest:** none declared.

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# Current Environment for Clinical Research with Medical Devices in Hospitals in Japan

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## Abstract

**Background:** Medical devices are continuously being improved in routine clinical practice. As necessary, new or additional clinical data for an investigational medical device is collected through clinical research and/or registered clinical investigations. We conducted a questionnaire survey to determine the current environment for clinical research with medical devices, particularly focusing on infrastructure and human resources in hospitals.

**Methods:** The questionnaire for this study included 6 main topics: experience of clinical research, in-hospital manuals, issues on clinical research, related regulations, and effectiveness of a guidance published by the Medical Engineering Technology Industrial Strategy Consortium. The questionnaire was mailed to all 10 core clinical research centers and 30 major clinical trial institutions at the time of survey in Japan.

**Results:** Eighteen hospitals (45%) provided responses. Relatively few clinical research activities with medical devices had been conducted in each hospital, and two-thirds of respondents thought low number of clinical research activities was problematic. A shortage of experts in medical devices was also raised as an important challenge. Most of the hospitals had established in-hospital manuals for clinical research with medical devices; however, specific features required for the evaluation of medical devices might not be included in the manuals. Many hospitals had too few clinical research coordinators (CRCs) for support of clinical research with medical devices, but half of the hospitals could not afford to increase the number of CRCs.

**Conclusion:** Our study revealed that the current environment for clinical research with medical devices in hospitals has been partly organized, but it was suggested that a shortage of experts, the complexity of the regulatory system, and a need for financial support are remaining issues.

**Keywords:** Medical device; Clinical research; Clinical trial; Questionnaire survey; Clinical research coordinator

## Introduction

Medical devices play key roles in diagnosis and treatment of diseases in modern healthcare. Unlike drugs, medical devices are continuously improved in routine clinical practice during the development and post-marketing phases to meet the needs of medical staff and patients. However, not all of advanced medical devices used in other countries are available in Japan [1]. Correction of this problem requires establishment of regulations related to development of medical devices and development or improvement of human resources, infrastructure and funding for clinical research and registered clinical investigations with medical devices. In this paper, clinical research is defined in a limited sense as research activities not including registered clinical investigations with Good Clinical Practice (GCP) for a marketing approval application.

During the development process, investigational medical devices are firstly evaluated based on clinical evidence including clinical data such as literature data and/or clinical experience. In response to the needs of new or additional clinical data, clinical research and/or registered clinical investigations are conducted. In particular, an innovative and/or invasive medical device for which clinical data are required for a marketing approval application under the Pharmaceutical Affairs Law (PAL) is evaluated in registered clinical investigations in accordance with the Ministry of Health, Labour and Welfare (MHLW)'s Ministerial Ordinance on GCP for Medical Device. Such clinical investigations are mostly sponsored by medical device companies. Once the safety and effectiveness of a medical device have been evaluated and ensured by the Pharmaceuticals and Medical Devices Agency, the regulatory authority in Japan, and subsequently approved by the MHLW, the medical device becomes accessible to medical staff and patients across the country. In contrast, clinical research with medical devices are predominantly initiated by clinicians in hospitals and generally conducted under permission of each hospital, in accordance with the Ethical Guideline for Clinical Research [2]. Clinical research assures timely evaluations of prototypes of medical devices with novel or altered technologies and with improved usability and/or performance.

In Japan, clinical research and registered clinical investigations are regulated separately and the system is complicated. Clinical investigations are regulated more clearly than clinical research and must be conducted in accordance with the PAL and GCP. Unapproved medical devices are regulated by the PAL, and therefore there was a concern that the supply of unapproved medical devices for clinical research conducted in hospitals constitutes a breach of the PAL. This background caused problems when companies make decisions on supplying medical devices to be tested in clinical research.

Recently, the MHLW released two notices regarding clinical

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research with unapproved medical devices [3,4]. These notices indicated that supply of unapproved medical devices for clinical research is exempted from the PAL. To clarify these notices, the Medical Engineering Technology Industrial Strategy Consortium (METIS) published a document entitled "Guidance on clinical research with unapproved medical devices" [5] to streamline the process of clinical research with medical devices. This guidance includes information on the overall picture of clinical research, overviews of related regulations, classification of medical devices, practical considerations at each stage of clinical research, checklists for protocol and informed consent form, and relevant templates for documents such as a collaborative research agreement.

This guidance further defined the regulatory requirements for clinical research with medical devices that are not regulated by the PAL or GCP; however, the current environment for conducting such clinical research in hospitals remains unclear. In Japan, individual hospitals are relatively small and are scattered nationwide, and this situation presents a barrier to efficient development of medical devices and drugs. Therefore, we conducted a questionnaire survey to determine the current environment for clinical research with medical devices, particularly focusing on infrastructure and human resources in hospitals, and to identify issues related to the conduct of clinical research from a hospital perspective.

## Materials and Methods

A questionnaire for this study was developed to examine the current environment for clinical research with medical devices in hospitals. The questionnaire included 6 main topics: experience of clinical research, in-hospital manuals, issues on clinical research, roles and sufficiency of support staff, related regulations, and effectiveness of the METIS guidance. The support staff refers to as Clinical Research Coordinators (CRCs) who support clinical research and/or registered clinical investigations. Most of the questions were multiple-choice for the purpose of reducing the time and effort of the respondents, but free descriptions were also obtained as necessary (Table 1).

The survey was conducted between 23 March and 25 April 2012. The questionnaire was mailed to directors of support offices for clinical investigations at all 10 core Clinical Research Centers (CCRCs) and 30 Major Clinical Trial Institutions (MCTIs) at the time of survey in Japan. The MHLW has designated these hospitals for financial support for human resources and infrastructure for smooth and efficient conduct of clinical research and registered clinical investigations [6]. It is particularly important to understand the current status of clinical research with medical devices in these hospitals since they have key roles in development of medical devices and drugs. Data were compiled using Microsoft Office Excel 2010.

## Results

Eighteen hospitals (45%) responded to our questionnaire, but some respondents did not answer all of the questions. The reported experience of clinical research with approved or unapproved medical devices in each hospital are shown in Table 2. Relatively few clinical research activities with medical devices had been conducted in the last 2 years. The median number of clinical research activities with medical devices was 5 per hospital when calculated with experience in 12 hospitals where had reported experience of at least one clinical research activity with medical devices, with considerable variation among the hospitals (range, 1-22 per hospital).

The results from the questions on preparation of in-hospital manuals for clinical research and registered clinical investigations with medical devices are shown in Table 3. Thirteen hospitals had established manuals for clinical research with medical devices, and 3 of these hospitals had manuals for clinical research in compliance with GCP. Manuals for clinical research with medical devices were the same as those used for drugs in 15 hospitals, and only 6 of these hospitals have manuals that cover clinical research with both approved and unapproved medical devices. Similarly, manuals for registered clinical investigations with medical devices were the same as those used for drugs in most hospitals.

There were several general issues on conduct of clinical research with medical devices (Figure 1). In particular, two-thirds of respondents thought that the much lower number of clinical research activities with medical devices compared to those with drugs was problematic. In this context, 4 respondents suggested that there was a shortage of experts in this field and/or indicated a lack of applicability of experience in clinical research with drugs due to methodological differences. There was an opinion that the low number of clinical research activities is one of the reasons why they could not hire staff specialized in medical device. Five respondents felt that separate management of investigational medical devices for clinical research and medical devices for routine practice was complicated. One of respondents' requests for medical device companies is more proactive technical support, for example, assistance on how to manage investigational medical devices.

The roles of CRCs were mainly to support registered clinical investigations in more than a half of the hospitals (Table 4). The median number of CRCs in each hospital was 7 (range, 2-18). Seven hospitals assigned a median of 2 CRCs (range, 1-5) as staff specialized in clinical research and registered clinical investigations with medical devices. Most respondents thought that more CRCs were needed in their hospitals, but half of the hospitals could not afford to increase the number of CRCs. These results suggest a common trend of an insufficient number of CRCs in the hospitals, particularly for support of clinical research with medical devices.

The notification on supply of unapproved medical devices issued by MHLW was highly recognized (16/18, 89%). Out of those who answered that they know the notification, 10 respondents agreed that clinical research will be more activated by the notification. To the question whether regulations should be eased so that unapproved medical devices can be provided to researchers at the request of companies, the respondents were almost equally divided between those who agreed and disagreed. In addition, out of 6 respondents disagreed that clinical research will be more activated by the notification, 4 respondents agreed to the aforementioned question whether regulations should be eased.

The METIS guidance was highly appreciated. The respondents thought the guidance was useful for physicians, dentists, CRCs and administrative officers associated with clinical research, including staff in charge of ethical review. The followings were listed as especially useful among the contents of the guidance: procedures for clinical research with medical devices, a template for a collaborative research agreement, classification of medical devices, and methods for management of investigational medical devices. In particular, the visual summaries shown as flowcharts and tables, including the overall picture of clinical research and the classification of medical devices, were highly praised. Some additions to the current guidance were proposed, including a template for a study protocol and methods for dealing with malfunctions of investigational medical devices.

| Topic | Question [Question type] | Response options |
|---|---|---|
| Experience of clinical research | Which and how much experience does your hospital have related to clinical research with medical devices, excluding experience related to registered clinical investigation? [Filling in numbers of all applicable experience within the last 2 years] | • Experience with approved medical devices within the approved indications<br>• Experience with approved medical devices under off-label use<br>• Experience with unapproved medical devices<br>• No experience |
| In-hospital manuals | Have manuals for clinical research been established? [Single answer] | • Yes / No |
| | If no, are manuals for clinical research in preparation? [Single answer] | • Yes / No |
| | Are the manuals for clinical research with medical devices the same as those used for drugs? [Single answer] | • Yes / No |
| | Are the manuals for registered clinical investigations with medical devices the same as those used for drugs? [Single answer] | • Yes / No |
| Issues on clinical research | Which issue do you face when conducting clinical research with medical devices? [Multiple answers allowed] | • Lack of applicability of experience in clinical research with drugs.<br>• Shortage or none of experts in medical devices in the hospital.<br>• More difficult to recruit participants compared to clinical research with drugs.<br>• Separate management of investigational medical devices for clinical research and medical devices for routine practice is complicated.<br>• Much lower number of clinical research activities with medical devices compared to those with drugs.<br>• Insufficient support from medical device companies. |
| | If any comments on cooperation from medical device companies, please specify. [Open-ended] | |
| | If any other issues, please specify. [Open-ended] | |
| Roles and sufficiency of support staff | What are CRCs in your hospital involved in? [Single answer] | • Only registered clinical investigations<br>• Mainly registered clinical investigations<br>• Both at about the same level |
| | Do you think the number of CRCs in your hospital is sufficient? [Single answer] | • Yes / No |
| | Does your hospital plan to increase the number of CRCs? [Single answer] | • Yes / No |
| Related regulations | Do you agree that clinical research will be more active thanks to the notification "Application of Pharmaceutical Affairs Law to supply of unapproved medical devices used in clinical research" issued on March 2010? [Single answer] | • Agree / Disagree / Do not know the notification |
| | Do you agree that regulations should be eased so that unapproved medical devices can be provided to researchers at the request of companies? [Single answer] | • Agree / Disagree |
| Effectiveness of the METIS guidance | What kinds of professional are likely to find the METIS guidance useful? [Multiple answers allowed] | • Medical doctor / Dentist / Nurse / Pharmacist / Other paramedics / CRC / Officer of clinical research center / Member of ethical committee |
| | If other, please specify. [Open-ended] | |
| | Which part of the METIS guidance is considered to be useful? [Multiple answers allowed] | • Classification of medical devices<br>• Differences in regulatory systems between non-registered clinical research and registered clinical investigations<br>• Procedures for clinical research with unapproved medical devices<br>• Preparation of protocol and informed consent form<br>• Templates of contracts<br>• Management of investigational medical devices<br>• Protection of participants in clinical research<br>• Interruption, cancellation, and termination of clinical research<br>• Check-lists for protocol and informed consent form |
| | If other, please specify. [Open-ended] | |

**Table 1:** Contents of Questionnaire.

## Discussion

Medical devices are continuously being improved in routine clinical practice. As necessary, new or additional clinical data for an investigational medical device is collected through clinical research and/or registered clinical investigations. To our knowledge, this report is the first survey of the environment for clinical research with medical devices in hospitals in Japan.

In-hospital manuals for clinical research with medical devices were established or in preparation at the time of the study; however, two major issues with these manuals were identified that might affect the quality of clinical research. The first is that some hospitals prepared the manuals in compliance with GCP. Clinical research with medical devices is not necessarily conducted in compliance with GCP under the current regulatory system, and the conduct of clinical research with such high level of quality is an overreach and a waste of time,

| | n (%) |
|---|---|
| With approved medical devices | 12 (67%) |
| Within the approved indications | 11 (61%) |
| Under off-label use | 9 (50%) |
| No reply | 5 (28%) |
| With unapproved medical devices | 9 (50%) |
| None or unknown | 5 (28%) |
| No reply | 4 (22%) |

**Table 2:** Experience with clinical research with medical devices in each hospital (N=18).

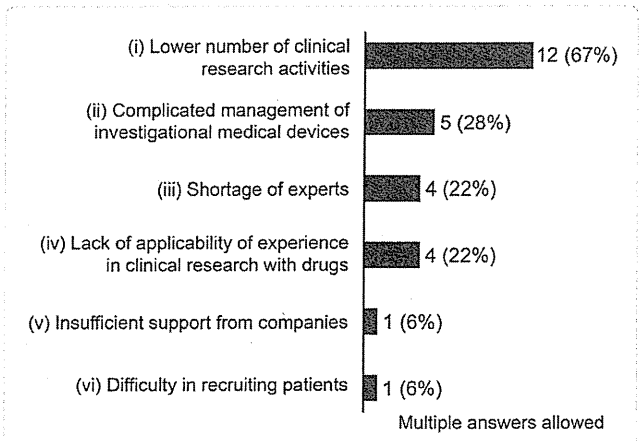


**Figure 1:** Issues on clinical research with medical devices.
(i) Much lower number of clinical research activities with medical devices compared to those with drugs.
(ii) Separate management of investigational medical devices for clinical research and medical devices for routine practice is complicated.
(iii) Shortage or none of experts in medical devices in the hospital.
(iv) Lack of applicability of experience in clinical research with drugs.
(v) Insufficient support from medical device companies.
(vi) More difficult to recruit participants compared to clinical research with drugs.

| | Yes | No | No reply |
|---|---|---|---|
| | n (%) | n (%) | n (%) |
| Have manuals for clinical research been established? | 13 (72%) | 4 (22%) [a] | 1 (6%) |
| Are the manuals for clinical research with medical devices the same as those used for drugs? | 15 (83%) | 1 (6%) | 2 (11%) |
| Are the manuals for registered clinical investigations with medical devices the same as those used for drugs? | 13 (72%) | 4 (22%) | 1 (6%) |

a: All the 4 hospitals had not established manuals for clinical research, but had been preparing at the time of the survey

**Table 3:** Manuals for clinical research and registered clinical investigations with medical devices (N=18).

money and effort of researchers and companies. The complexity of regulatory systems might underlie this problem. Different regulatory systems are applied separately to clinical research and registered clinical investigations [7,8]. A possible solution may be to unify the two systems for one system like investigational device exemption in the United States.

The second issue is that the manuals for clinical research, as well as registered clinical investigations, with medical devices were the same as those used for drugs in most of the hospitals (Table 3). This implies that specific features required for the evaluation of medical devices are not included in the manuals. The respondents indicated substantial differences in procedures in clinical investigations and clinical research with drugs and medical devices, and experience in clinical research with drugs cannot always be applied to medical devices (Figure 1). This issue may arise from insufficient experience with clinical research and clinical investigations with medical devices; thus, specific procedural descriptions might not be included in the in-hospital manuals.

Relatively few clinical research activities with medical devices had been conducted in each hospital. Therefore, the experience and findings from clinical research with medical devices should be shared among hospitals and medical device companies to improve development of medical devices to the extent possible. The METIS guidance will be updated based on the needs of medical staff and medical device companies and on changes in the environment for medical device development. The updated guidance is expected to include some case studies and more specific procedural advice, which should partly complement the knowledge and experience in hospitals and companies.

A shortage of experts in medical devices was raised as an important challenge (Figure 1). In Japan, the delay of clinical research and clinical investigations with drugs and medical devices following basic research is often pointed out [9]. In particular, the characteristics of medical devices vary widely and multidisciplinary knowledge is needed in medical device development. A recent comparison of undergraduate and graduate education at universities in Japan and the United States for development of human resources for promotion of development and application of medical devices led to several proposals [10]. These included continuous funding for the centers of excellence in research and education as necessary, quality control of educational programs, accreditation for educational programs, and strengthening of regulatory science education. Such education can also enhance the effectiveness of on-the-job training and achieve flexible application of knowledge.

There are several limitations that affect the validity of the study. We sent the questionnaire to all CCRCs and MCTIs designated by the MHLW at the time of the survey, but the response rate was only 45% and some respondents did not answer all of the questions. An unbalanced distribution of non-respondents and respondents limits the internal validity of the study. Generalizability of the study may also be limited because CCRCs and MCTIs are highly organized compared to most hospitals in Japan. Further studies need to include smaller hospitals because innovation in medical devices can occur anywhere. In addition to the issues raised by the present study, other challenges may exist in medical device development in Japan, as discussed in the United States [11]. Issues and challenges will vary with changes in the regulatory system and accumulation of experience in medical device development

| | n (%) |
|---|---|
| What are CRCs in your hospital involved in? | |
| - only registered clinical investigations | 2 (11%) |
| - mainly registered clinical investigations, but also clinical research | 11 (61%) |
| - both registered clinical investigations and clinical research at about the same level | 4 (22%) |
| No reply | 1 (6%) |
| Do you think the number of CRCs in your hospital is sufficient? | |
| - sufficient | 2 (11%) |
| - insufficient, but plans to increase | 8 (44%) |
| - insufficient, and no plan to increase | 7 (39%) |
| No reply | 1 (6%) |

**Table 4:** Roles and sufficiency of clinical research coordinators (CRCs) (N=18).

in hospitals and companies, and these should be identified and resolved on an ongoing basis.

In conclusion, our study revealed that the current environment for clinical research with medical devices in hospitals has been partly organized, but it was suggested that a shortage of experts, the complexity of the regulatory system, and a need for financial support are remaining issues. Measures to meet these challenges should be taken to create a positive cycle of medical device development.

## Conflict of Interest

All authors declare no conflict of interest with regard to this work.

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# 第8章　製品種別ごとのデータ・情報の取得とまとめ方のポイント

## 第5節
## 核酸医薬，遺伝子治療薬，細胞治療薬における留意点

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(株)技術情報協会
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上手な書き方・まとめ方～審査に不可欠なデータ・情報の取得の仕方～」抜刷

## 第5節　核酸医薬，遺伝子治療薬，細胞治療薬における留意点

### 1. バイオ医薬品の規格設定と検査方法

バイオ医薬品の開発にあたり，臨床試験の実施にあわせた試験物の性質や製造に関する chemistry, manufacturing, and control (CMC) の整備にあたっては，特に米国ではフェーズ 1cGMP の精神が存在していることを理解することは重要である。

20 世紀末からのバイオテクノロジー技術の臨床応用における急速な進展によって，疾患にかかわる特異的標的をターゲットとした抗体医薬，遺伝子治療，治療的ワクチンなどの生物製剤（バイオテクノロジー医薬）の研究開発が増加した。米国においては，FDA が臨床試験審査の拠りどころとする Investigational New Drug (IND)application 制度の中で，バイオテクノロジー技術を応用した創薬を行う大学等アカデミアの研究機関や小規模の製薬企業の施設，資金，経験，知識では，市販後製造を念頭においた 1978 年 9 月の医薬品・バイオ医薬品に関する cGMP 連邦行政規則（21CFR 210/211），あるいは 1991 年の Guidance on the Preparation of Investigational New Drug Products (Human and Animal) への対応が困難になってきていた。特にバイオ医薬品はその剤型の新規性や安全性・有効性両面での大きなチャレンジもあることから，フェーズ 1 にはいっても必ずしもフェーズ 3 を終了して上市できない。そのため探索医療の範疇にあるフェーズ 1 においては，小規模の研究開発投資で医薬品候補物質を製造して，健常人（あるいは患者）における反応性を確認していくという必要性が生じてきた。そこで，CMC 審査も，フェーズ 1 に対する cGMP 基準は，被験者の安全性を担保するために最低限の科学的妥当性をどのように評価するのかということに力点が置かれてきた。

このような審査の現場の考え方をまとめた形で，2006 年 1 月，米国 Food and Drug Administration (FDA) の Center for Drug Evaluation and Research (CDER) および Center for Biologics Evaluation and Research (CBER) から "Guidance for Industry: INDs - Approaches to Complying with CGMP During Phase 1"（フェーズ 1cGMP）がドラフトガイダンスとして発表された。本ガイドラインは 2008 年 7 月に最終版となっている。CDER と CBER の連名となっていることからわかるように，低分子化合物などのみならずバイオ医薬品をも対象として，フェーズ 1 臨床試験の試験物質（治験薬）製造の cGMP に関する当局の考え方を広く示したものである。フェーズ 1cGMP ガイダンスでは，フェーズ 1 に対する cGMP は，被験者の安全性を担保するために最低限の科学的妥当性をどのように評価するのかということに力点が置かれている。特に新規性の高い治療方法の first in human 試験を考慮する場合には，この精神を理解することは重要である。

バイオ医薬品の CMC は，タンパク製剤，遺伝子医薬，細胞医薬など多岐多様にわたることから，一元的に解説することは困難である。そこで，以下に，いくつかの例についての臨床試験の実施に必要な基本的な要点を記載する。ただし，承認申請 (Biological License Application; BLA) にかかる CMC は，抗体医薬，タンパク製剤以外はまだ殆ど事例がないことから，行政当局と協議をして慎重に行っていく必要があろう。

### 2. 遺伝子医薬・核酸医薬

遺伝子治療に用いられる遺伝子医薬の CMC に関しては，2004 年 11 月に Guidance for FDA Review Staff and Sponsors: Content and Review of Chemistry, Manufacturing, and Control Information for Human Gene Therapy Investigational New Drug Applications (Draft Guidance) が発表されている。とくにベクターおよびセルバンクの安全性と規格，その他製造に使用する試薬についての安全性が必要となる。製造と精製にあたっては，最終産物の構成，保存方法，安定性，そして安全性と品質を確認するための各段階での分析に十分に考慮することが肝要である。

安全性に関しては，清潔度，感染性の細菌やウイルスの否定，エンドトキシンレベルの測定やマイコプラズマの否定が必要である。規格に関しては，identity としてシークエンスや構造，純度（細胞由来の核酸やタンパク，その他試薬の混入を測定），安定性，またフェーズ 2 終了時までに，生物学的試験によって臨床での効果を *in vitro* で予測するための potency 試験も整備することが望まれている。マスターセルバンクについても清潔度，感染性の細菌やウイルスの否

定が必要であるが，とくに人由来細胞を使用している場合には，人の感染性病原体（EBV, HBV, HCV, CMV, HIV 1&2, HTLV 1&2, AAV, B19），マウス由来細胞の場合にはMAPテストを実施する。ワーキングセルバンクにおいては，マスターセルバンクにおける測定項目のうち特に重要なものを選択して実施する。

RNAi，アプタマー，アンチセンスなどの核酸医薬の場合は，基本的に合成で製造できることから，低分子化合物に準じて物理化学的性質を規定していくことが可能であると考えられる。構造の同定は，分子量，塩基配列，ナトリウム量，構造決定，ヌクレオチド間の連結，Tm（2本鎖解離温度），塩基鎖長，2本鎖・1本鎖含有量といった項目によって実施する。

## 3. 細胞医薬

細胞医薬は，再生治療や癌ワクチンなどに使用されることが多い剤型である。通常は人（自己あるいは同種）由来の細胞を修飾して使用されるため，まずは細胞の採取と感染のコントロールが重要となる。米国FDAは2001年から，細胞組織利用製品の施設登録，細胞組織利用製品をリストアップするための統合システムの作成，危険因子のスクリーニングと感染症検査結果に基づいたドナー組織，細胞などの適格性確認の基準の規定などを進めてきた2005年5月にcurrent Good Tissue Practice (cGTP) ガイドラインの最終案を施行し，米国内の細胞組織利用製品の製造業者に対し，感染症の感染や感染拡大を予防するための採取，処理，保存，ラベリング，パッケージング，搬送のための規定と，記録管理の手順などを制定した。cGTPとcGMPとでは，それぞれの規制対象の違いから項目の内容は若干異なってはいるが，製造に関する主要事項（人員，環境，記録，安全性）については共通して項目が設けられている。しかしながら，cGTPでは試薬，製品について公衆衛生法に基づいているかどうかの適合性を要求しているのに対してcGMPでは該当する項目はない。さらに，cGTPでは細胞組織利用製品の使用後についても追跡が可能となるように個別化，追跡記録について明記していること，そして，これらのcGTPについてFDAの査察および相談ができることが定められている。

細胞医薬のCMCについては，2003年8月にGuidance for Reviewers: Instructions and Template for Chemistry, Manufacturing, and Control (CMC) Reviewers of Human Somatic Cell Therapy INDsのドラフトガイダンスが発表されている。項目のみかいつまんで記載すると，

・規格について

（1）細胞ソース

自己由来か同種由来か，細胞ソース，修飾プロトコル，採取方法，ドナースクリーニング，病原体検査

（2）細胞バンクシステム

マスターセルバンク (MCB)，ワーキングセルバンク (WCB)，安全性，アイデンティティ，純度，安定性，細胞の活性度，培養条件，保存条件，継代後のフェノタイプの安定性

（3）試薬

最終製剤に含まれないこと（FBS，トリプシン，成長因子，サイトカイン，抗体，抗生物質など），由来，品質保証 (CoA)

・製造について

（1）細胞の準備

採取方法，閉鎖系システムか否か，放射線により増殖不能にしても必要な特性を維持しているか，ひとつひとつのプロセスにかかる時間

（2）最終段階での回収

遠心，洗浄の状態と方法など

（3）最終製剤の組成

細胞の濃度，運搬データなど

・細胞の評価方法について

（1）微生物の混在

感染性試験の実施，試験時期，マイコプラズマ，外来性病原体については*in vitro*（ウイルスによる細胞感作），*in vivo*（マウス，卵）

(2) 細胞医薬としてのアイデンティティ

複数の細胞が使用されている場合は区別が必要，細胞表面マーカー，遺伝子多型

(3) 純度

製造に使用した試薬の混在，エンドトキシンレベル（Pyrogenicity；＜5EU/kg 体重 /dose）

(4) Potency

相対的生物学的機能の評価，フェーズ2終了時までに測定法を開発すること

(5) その他

細胞のバイアビリティ（＞70%），細胞数（ドーズ）の最小量，最大量とその理由

# 分子標的薬・コンパニオン診断薬の医療技術評価の現状と課題

Health Technology Assessment(HTA)of molecular targeted drugs and companion diagnostics

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◎分子標的薬の開発が進むとともに，今後ますます個別化医療は加速し，薬剤は有効な集団にのみ与えられるようになっていくであろう．分子標的薬の開発とともに，この“有効な集団”を絞り込むためにコンパニオン診断薬も開発される．昨今，分子標的薬およびコンパニオン診断薬の医療技術評価(HTA)についての議論が行われている．HTAは医療技術を医学的・社会的・倫理的・経済的な観点などから総合的に検討するものであり，評価の結果は新薬や新技術などの保険償還の可否や償還価格の設定，臨床ガイドラインの策定などへの使用が期待されている．日本では分子標的薬・コンパニオン診断薬のHTAはまだほとんど行われていないが，医療保険制度の枠組みのなかで，このような医療を効率的に提供していくためにはHTAが不可欠であり，その適用範囲や社会の受容といった種々の課題に対して幅広い議論が求められる．

Key word：分子標的薬，コンパニオン診断薬，医療技術評価(HTA)

日本では国民皆保険による公的医療保険制度が構築されていることから，効率的な医療を提供することが求められる．個別化医療は，より安全で有効な先端技術の利用，医療の質の向上やむだの削減のために重要視されている[1]．分子標的薬の開発が進むとともに，今後ますます個別化医療は加速し，薬剤は有効な集団にのみ与えられるようになっていくであろう．分子標的薬の開発とともに，この“有効な集団”を絞り込むためにコンパニオン診断薬も開発される．

昨今，分子標的薬およびコンパニオン診断薬の医療技術評価(health technology assessment：HTA)についての議論が活発に行われるようになってきており，本稿ではわが国における現状と課題を中心に概説する．

## 医療技術評価(HTA)

HTAは，エビデンスに基づく医療(evidence based medicine：EBM)や比較効果研究(comparative effectiveness research：CER)より一歩進んだ概念であり，“その治療は効果があるのか”“何がもっとも効果的に機能するのか”に加えて，“その治療を受ける価値があるのか”という問いにまで答えようとするものである[2]．つまり医療技術を医学的な観点だけではなく，社会的・倫理的・経済的な観点などから総合的に評価するものである[3]．

その評価手法は疫学，生物統計学，行動科学といったソリッドな科学に基づいており，1990年代後半からヨーロッパ，アメリカ，アジア諸国においてHTAを実践する独立機関が設立されている．これらの国では，HTAの結果は新薬や新技術などの保険償還の可否や償還価格の設定，臨床

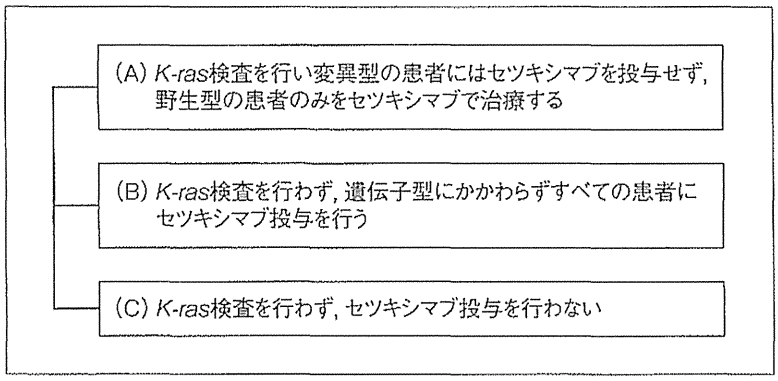


図 1　白岩らの研究で用いられた比較対照群

ガイドラインの策定などに用いられている．日本においては，国民医療費が増加しつづけているという状況のなか，2012 年 5 月に厚生労働省の諮問機関である中央社会保険医療協議会に費用対効果評価専門部会が設置され，医療技術の保険適用などの決定に費用対効果の観点を導入することについての議論が開始された[4]．

コンパニオン診断薬においては，2011 年 10 月に社団法人日本臨床検査薬協会，米国医療機器・IVD 工業会および欧州ビジネス協会が，内閣官房の医療イノベーション推進室，厚生労働省および独立行政法人医薬品医療機器総合機構に向けて“個別化医療を推進するためのコンパニオン診断薬のインフラ整備に関する提案書”を発出し，検査の技術革新や製品開発へのインセンティブを加算した保険点数付与の仕組みの導入や，コンパニオン診断薬の医療経済的な価値を反映した保険点数の付与を要望している[5]．

## 診療報酬上のコンパニオン診断薬の価値

コンパニオン診断薬を含む体外診断薬の診療報酬は検査の実施料と判断料からなり，その保険点数には検査キット代だけではなく，測定にかかる人件費や検体前処理，輸送，報告など，検査にかかるすべてのプロセスに対する費用が含まれている[6]．これまで，遺伝子検査の診療報酬は 2,000 点が上限と考えられており，その技術や臨床的有用性を反映した償還価格の付与が行われがたい仕組みであった[5]．2010 年 4 月に 2,000 点で保険収載がなされた *K-ras* 遺伝子検査キットについては，保険収載後，8,000 点に設定したとしても費用対効果が優れるという研究結果が示されたが[7]，2012 年度診療報酬改定ではわずかな増点(2,100 点)にとどまった．

一方，2012 年 3 月に承認されたコンパニオン診断薬については従来の遺伝子検査などの診療報酬である約 2,000 点を大きく上まわり，非小細胞肺癌に対する *ALK* 融合遺伝子標本作製(fluorescent *in situ* hybridization：FISH 法)には 6,520 点，再発または難治性の成人 T 細胞白血病リンパ腫に対する CCR4 蛋白の検出(免疫組織化学染色法および flow cytometry 法)には 10,000 点という点数が付けられており[8,9]，今後はコンパニオン診断薬に診療報酬上で高い価値が与えられる事例が増えると予想される．しかし，これまで実施されていなかった新技術の革新性を適切に評価することは難しい．また，市場ニーズが低いが臨床的に価値が高い場合においても，コンパニオン診断薬の適切な償還価格を算出することはきわめて難しい[10]．

## 分子標的薬・コンパニオン診断薬の経済評価

ヨーロッパにおいて HTA はさまざまな医薬品に適用されてきたが，コンパニオン診断薬を含む診断薬ではほとんど実施されていない[11]．このようななか，2013 年 8 月に HTA が進んでいるイギリスにおいて，HTA を所管する行政機関である National Institute for Health and Care Excellence(NICE)が，費用対効果が優れていると評価することができないとして，*ALK* 融合遺伝子陽性の非小細胞肺癌の治療に ALK 阻害薬であるクリゾチニブの使用を推奨しないとするガイダンス[12]を公表し，衝撃を与えた．