

Combined Bio-Prosthesis for Oesophagus Defect Repair (Experimental Study)

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Abstract

Background: Up to now repair of oesophagus (defects/stenosis) remains a major problem despite significant technical progress in surgery. The purpose of this work was to test the use of chitosan scaffolds and foetal digestive tract implants to repair cervical oesophagus defects.

Material and Methods: The experiments were conducted on 158 laboratory rats and 33 mice, according to the Bioethics rules. In a first part, chitosan was tested as a biocompatible and degradable scaffold in *in vitro* and *in vivo* experiments. In a second part, both oval (patch) and circular (segmental) oesophageal defects were repaired using chitosan tubes alone or chitosan scaffold reinforced by a flap of grown foetal oesophageal implant. Suture alone or grown foetal organ implants alone were used as control. Imaging and histology of the repaired region and of other organs were regularly performed up to 13 months post-surgery.

Results: Chitosan tubes with a 2-week degradation time in alkaline medium (at pH 7.1 - 7.4) were used for combined bio-prosthesis in oesophageal defect repair. In oval defects, chitosan tubes alone were enough to obtain favourable outcomes and fast oesophagus wall restoration. In segmental defects, the best results (survival, recovery delays, absence of stenosis or diverticula formation) were obtained by combining the chitosan tube with a well vascularized flap from grown foetal oesophagus implant or neck muscle. The factors of success were: tight adaptation of the tubular chitosan scaffold to the oesophagus wall, optimizing the mucosa and submucosa regeneration (no circular ligature), reinforcement of the gap by a vascularized flap, adequate early post operation management.

Conclusion: Combined bio-prosthesis - chitosan scaffold with a vascularized flap of developed foetal digestive organ implant was the most suitable solution for circular oesophagus defect repair. Key factors seem to be: a prosthesis degradation after the first week and optimal conditions for regeneration of the mucosa and the muscular layers. If the use of foetal organ implant in clinics remains problematic, reinforcement of a chitosan tube by a vascularized muscle flap may be considered.

Keywords: Bio-Prosthesis; Chitosan; Foetal Organ Implantation; Oesophagus Defect Repair; Oesophagus Surgery; Oesophagus Histology; Oesophagus Imaging

Abbreviations

BW: Body Weight; CD: Circular Defect; Ch: Chitosan; FI: Foetal Intestine or Foetal Implant; FOC: Foetal Oesophagus Cyst; FOF: Foetal Oesophagus Flap; FSOE: Foetal Stomach-Oesophagus; HES: Haematoxylin Eosin Saffron; MF: Muscle Flap; OD: Oval Defect

Introduction

Repair of oesophageal defects remains, up to now, a problem in digestive surgery: morbidity and mortality after this kind of interventions remain high, i.e. up to 45% [1-11]. In the past, trials of using artificial prosthesis for repairing oesophagus defects after resection have been reported, but were unsuccessful [12-16]. This can be explained by two conflicting situations preventing the prosthesis to be

efficient and to be integrated into the organism. First, the necessity of waterproofing the gap does not allow the use of porous prosthesis structures, hence there is no possibility for surrounding cells to colonize the prosthesis. Second the prosthesis can be included into the organism only if it is covered on both surfaces by living tissues which is not possible for the oesophagus and generally for any hollow organs (contrary to blood vessels and heart). For these reasons such prosthesis gave many complications such as ulcers of the oesophagus wall, if they remained in place, or stenosis at the repair localization if they migrated into the stomach.

The concept of combined bio prosthesis composed of a polymeric scaffold and living cells for repairing oesophageal defect was born in the twentieth century [17-19]. However, the development of such combined bio prosthesis became possible only later on with the progress of the stem cell research [20-36].

As an alternative to *in vitro* stem cell culture, syngeneic foetal organ transplantation can be used for the formation of adult-like organs *in vivo* according to ontogenesis patterns [20,29-31,34]. Engineering of oesophagus as well as trachea was proposed. The results were encouraging for trachea [24-26], probably because trachea contains cartilage, which may be replaced by an artificial rigid structure conferring stability to the whole construction and keeping a lumen for air transit. This is not the case for oesophagus. This justifies the need for designing a waterproof tube ensuring the water and food transit, with a degradation rate corresponding to the formation of a new adequately structured and functioning oesophagus wall.

During the last past years, chitosan was one of the most extensively studied natural biopolymers in the field of biomedical research and pharmaceutical industries. Its use for topical, mucosal, external applications or even regenerative colorectal surgery has been largely described [34-40].

Purpose of the Study

The purpose of this work was to elaborate a technique of bioengineering oesophageal defects using chitosan tubes as a possible bio-compatible, well tolerated and efficient scaffold and foetal digestive organ implants for improving the regenerative process.

Material and Methods

Different chitosan scaffolds were used, all made of non-animal derived chitosan provided by KitoZyme (Belgium):

1. Porous membranes and porous tubes, prepared by Kitozyme (Belgium) with double porous layer (large pores for external layer to allow colonization by surrounding tissues) 1 - 1.5 mm wall thickness, 3 mm diameter (Figure 1A) and
2. Non-porous tubes produced by "Medovent" (Germany) with 0.2 - 0.3 mm of wall thickness and 1.5 - 2 mm of external diameter for insuring both stiffness and liquid transit (Figure 1B). Some of them were made radio opaque to follow up the *in vivo* degradation.

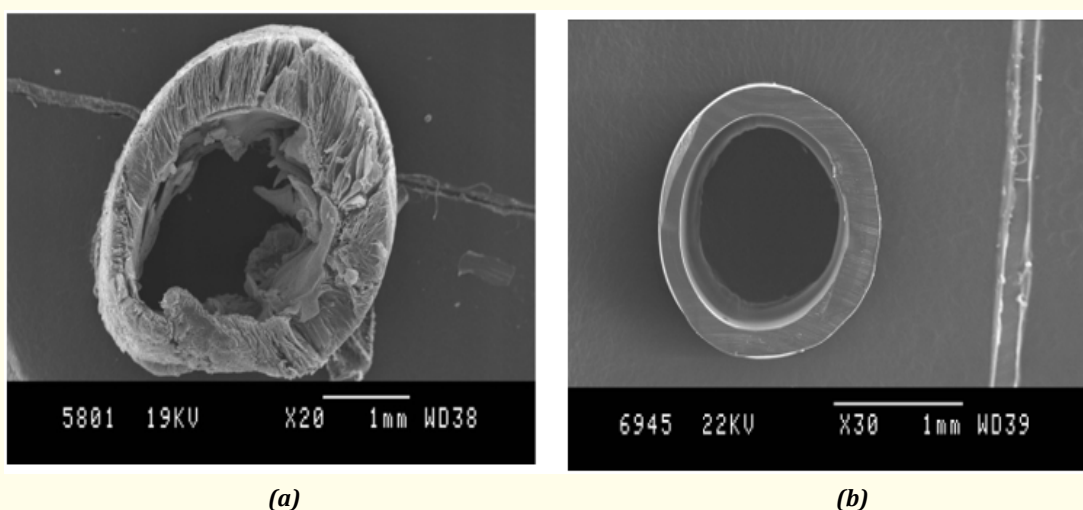


Figure 1: Scanning electron microscopy of chitosan tubes: porous bi-layer tube (A), non-porous tube (B).

Experiments were conducted on 158 white laboratory rats (Wistar and Fischer), and 33 mice (C53Bl), both males and females, aged 3 - 4 months at the beginning of the study. Animals were managed according to the rules of Bioethics and the experiments were agreed by the local Ethic Committee (protocol N° 50N).

Experiments were divided in part I and part II.

Part I was devoted to *in vitro* and *in vivo* experiments for studying biodegradation and biocompatibility of the chitosan scaffolds.

Part II was constituted by *in vivo* experiments aiming at oesophageal defect repair using chitosan tubes alone or combined with biological materials.

The first series of Part I experiments were conducted *in vitro*. Chitosan membranes of the same size, i.e. 10 x 5 x 1 mm or non-porous chitosan tubes of 2 mm diameter, 20 mm length and 0.2 mm thickness were placed into 10 standard laboratory tubes of 1cm diameter filled a/with water, saline solution (5 ml) at pH varying from 1 to 10, or b/with saliva (5 ml) collected from either fasting or non-fasting people- pH being, respectively, 6.6 or 7.5 (Table 1A). In all these experiments duration and features of the chitosan degradation were recorded.

Series	Number of tests*	Observation delays	Type of scaffolds
Influence of pH	6	Up to 48 hours	Membranes
Influence of saliva	5	Up to 48 hours	Membranes and non-porous tubes
Total	11		

Table 1A: *In vitro* degradation tests on chitosan membranes and tubes.

*: Each test included 10 laboratory tubes.

The second series of Part I included experiments conducted *in vivo* on mice and rats to test the reaction of tissues to chitosan implants and to evaluate the *in vivo* degradation rate of the chitosan membranes and tubes (Table 1B).

Series	Implants	Animal Number	Implant Site	Implant Number	Investigations	Observation delays
1	FI	52 rats	Ear	104	Visual, Histology	Up to 6 months
2	Paraffin film	5 mice	Ear	10	Visual, Histology	2 months
3	Paraffin film + FI	5 mice	Ear	10	Visual, Histology	2 months
4	Polyethylene film	5 mice	Ear	10	Visual, Histology	2 months
5	Polyethylene film + FI	5 mice	Ear	10	Visual, Histology	2 months
6	Chitosan membrane or tube	8 mice	Ear	16	Visual, Histology	2 months
7	Chitosan membrane or tube + FI	5 mice	Ear	10	Visual, Histology	2 months
8	Chitosan tube	5 rats	Neck	5	Roentgen imaging	2 months
9	Chitosan tube + FI	3 rats	Neck	3	Roentgen imaging	2 months
Total		60 rats 33 mice		178		

Table 1B: *In vivo* comparative study of different material biocompatibility and biodegradability including foetal organ implantation (FI).

After anaesthesia by gas induction and intra peritoneum injection of a standard Nembutal® solution (0.075 mg/100g BW), a skin incision of 5 - 10 mm was made and chitosan material was introduced between the anterior muscles of the neck or under the skin of the ear pavilion. In groups 7 and 9, a piece of foetal intestine, stomach or oesophagus was also introduced in combination with the chitosan material.

Investigation methods were: Visual observation, Roentgen imaging of radio opaque chitosan tubes [39] and histology of the probes (hematoxylin-eosin-saffron staining and polarized light examination) at different stages of the material disaggregation.

The experimental design of part II and the schemas of surgical techniques used in are presented in table 2 and figure 2.

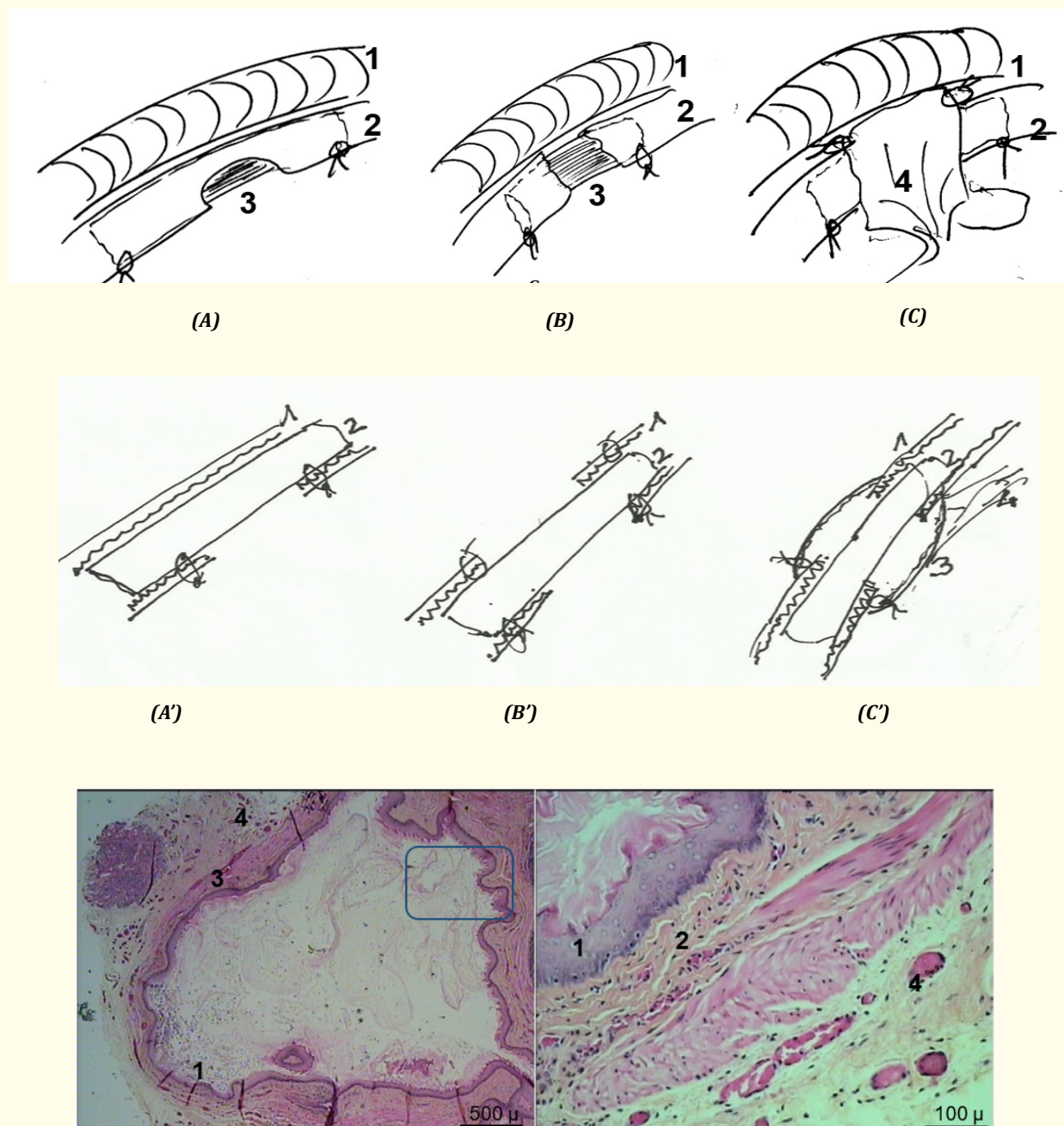


Figure 2: Schemas of surgical techniques for complex bioengineering of oesophageal defects.

A: Use of chitosan tube for oval defect repair; B: Use of chitosan tube for oesophagus segment defect repair;

C: Use of chitosan tube combined with vascularized flap of grown foetal oesophageal implant or of neck muscle.

Above (A-C): General view of trachea and repaired oesophagus: 1. Trachea, 2. Oesophagus, 3. Chitosan tube,

4. Vascularized flap. Below (A'-C'): Longitudinal section of the repaired oesophagus: 1. Oesophagus wall; 2. Chitosan tube;

3. Muscle or grown foetal oesophagus flap; 4. flap vascularized peduncle. D: Histology of the grown foetal oesophageal implant - cyst:

1. lumen and mucosa, 2. submucosa and 3. muscle layer, 4. external conjunctive tissue layer (HES).

Series	Rat Number (n)	Investigation	Observation delay
Chitosan tube alone	18	Histology	0 - 180 days
Chitosan tube + FOC flap	6	Histology	0 - 270 days
Total	24		

Table 2A: Oval oesophageal defect repair.
FOC: Foetal Oesophagus Cyst.

Series	Animal Number	Investigation	Observation delay
Chitosan tube alone	23	Histology	Up to 11 month
Chitosan tube + FOC flap	24	Radio, Histology	Up to 13 months
Chitosan tube + striated muscle flap	5	Histology	Up to 10 months
Only FOC flap	6	Histology	0 - 8 days
End-to-end suture	10	NA*	0 - 6 days
Intact control	6	BW evaluation	0 - 60 days
Total	74		

Table 2B: Circular (segmental) oesophageal defect repair.
NA: Not applicable

Table 2: Experimental design for part II.

After anaesthesia by gas (Isoflurane 4%) induction and intra peritoneum injection of a standard Nembutal® solution (0.075 mg/100g BW) and a subcutaneous injection of Atropine sulfate 1% 0.2 ml and after disinfection of the anterior surface of the neck, a median longitudinal incision of the skin was made, neck muscles were separated and the oesophagus neck part exposed.

According to the series, an oval defect of 2 x 3 mm was created with scissors (Table 2A) or a 4 +/- 1 mm long segment of oesophagus was resected (Table 2B). The main series were performed with the use of chitosan tubes combined or not with bio-material for oesophagus repair (see table 2B). In 10 control animals, the edges of the resected segment were sutured with 7° nylon or Ethilon® suture (end-to-end waterproof sutures). In 6 other control animals, the oesophagus defect was repaired by FOC only.

When a chitosan tube was used as scaffold for repair technique, this tube was introduced into the oesophagus by endoscopy-like procedure before creating the defect. The tube of 2 mm diameter tightly adhered to the wall and was fixed in place by 4 Ethilon® 6° stiches (Figure 2A, A', B, B').

To reinforce the anastomosis and enhance the oesophagus wall regeneration, in some animals (See table 2) a first surgical intervention was performed 2 - 4 months before the main one: a foetal oesophagus from a donor (aged 18 - 19 days of intra uterine development [20]), of the same strain to avoid rejection (syngeneic transplantation), was implanted between the anterior neck muscles. Its development was assessed visually (tumefaction of the neck) and by ultrasound investigation (See [38] and figure 2D).

In these cases, during the main operation, a vascularized flap of the developed cyst was fixed using 2 - 3 Ethilon 6° stiches to the edges of the oesophagus defect (Figure 2c).

In 5 animals, a flap from sterno-cleido-mastoid muscle (section at the sternum insertion, side mobilization but proximal end secured with good vascularization) was used as chitosan tube reinforcement. The cut edge of the muscle was fixed to the proximal and the distal part of the sectioned oesophagus using two Vicryl or Ethilon 6° stiches.

The operation site was closed by two layers of separate stiches (Vicryl 6°) for the muscles and four for the skin).

After surgery, the animals received only liquids during the first four days, condensed sugar milk till 28 - 30 days and, from day 14, they also received their usual pellets for breeding, ("Safe", France). To avoid ingestion of feces or litter (straw, saw particles that were found included into the new formed oesophagus wall in the first preliminary experiments), the animals were kept on grids during at least 2 months.

The post-surgical follow up was as follows:

- During the first fifteen days, the animal condition was daily evaluated and their BW measured. Later it was performed once a week.

Ultra sound investigations were realized by Siemens apparatus with a 17MHx probe (Siemens, Germany).

Roentgen evaluation of the presence of the tube and later the quality of the food transit was provided using Roentgen table (General Electrics "Prestige" NH, USA). The contrast employed for food transit investigation was Gastrografin® solution. Investigations were performed at 7 - 15 days and at 6, 10 and 13 month delays [39]. In rats sedated but with a deglutition reflex conserved, a catheter size 16 fixed on a 10 ml syringe was introduced in the mouth between jaws and cheeks and the solution was mildly propelled till the stomach began to fill. Serial imaging was performed.

At days 13, 25 - 35, 48, 60 and months 4, 6, 9, 11, 13, and after euthanasia (lethal overdoses of Nembutal) histology of intact and operated oesophagus, liver and kidney of the animals was performed. Samples were fixed in formalin 12% for 24 - 48 hours, embedded in paraffin. Hematoxylin-eosin-saffron stained slices of 3 - 4 µm thickness were examined in usual and polarized light.

Results

Part I

In vitro chitosan porous membranes and tubes degradation depended on the medium pH.

In water or saline (pH about 6), dislocation and fragmentation of the membrane structure started after 4 - 6 hours and was complete after 24 - 48 hours (Figure 3A). Similar observations were made with chitosan tubes, either porous or non-porous, with a significant shape alteration after 24 hour exposition to saline. Acidification of the medium (either water or saline) by addition of 0.1M acid acetic led to quick and complete degradation of the chitosan sample (within 1 hour), whereas addition of 0.1M NaOH to the same medium (pH 7.1 - 7.4) allowed to maintain the sample during 5 - 9 days.

In saliva which contains enzymes and whose pH varies from 6 to 7.4 depending on the digestion phase and the type of ingested food, the degradation process was even faster; i.e. exposition for 4 hours was enough to observe changes in chitosan tube color and shape while complete degradation occurred after 3 - 4 days by transversal splitting in case of non-porous tubes (Figure 3B).

In vivo experiments has shown that chitosan is perfectly biocompatible with surrounding tissue and with the growth of foetal organ (oesophagus, stomach or small bowel) implanted into a subcutaneous pouch of the ear pavilion in rat. Two months after surgery the chitosan degradation was in its terminal phase whereas the oesophagus-stomach implant was well developed (Figure 4A). The degradation of chitosan was accompanied by a mild and temporary inflammatory reaction that did not affect the implant. Chitosan scaffolds implanted in the ear or in the neck site were well integrated in the site and infiltrated by the surrounding cells without any pathologic reaction. At day 7 the gap between host tissue and chitosan tube, that was clearly visible (Figure 4B). It completely disappeared with time, and the surrounding cell penetration into the membrane was observed.

No anomaly of other investigated inner organs (lungs, kidneys, liver) was observed after chitosan use.

Implantation of radio opaque chitosan tubes under the skin of neck in rats (pH 7.4) have shown a good maintenance of the dimensions and shape of the prosthesis even 2 months post- surgery (Figure 4C).

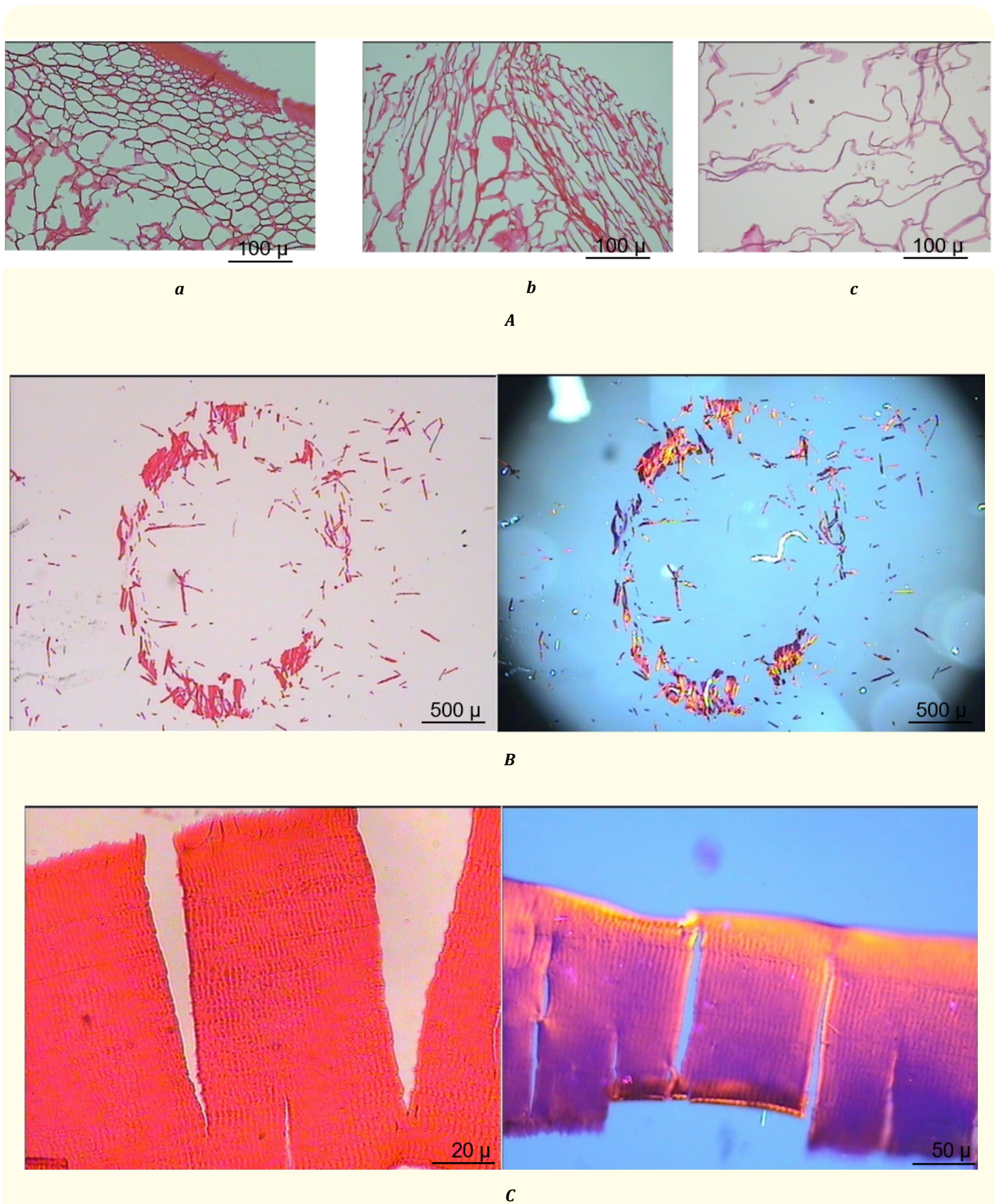
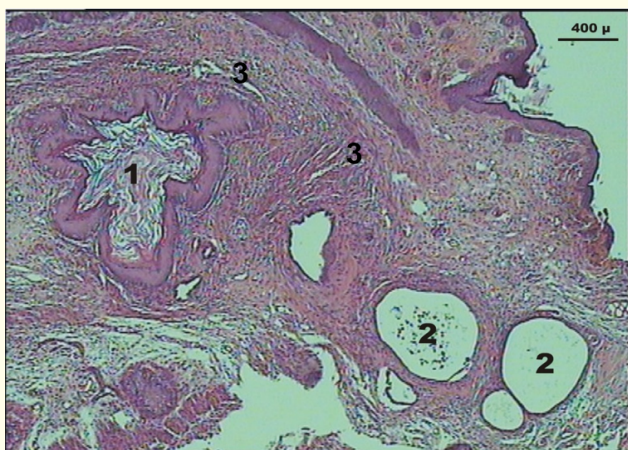


Figure 3: Chitosan membrane and tube structure observed by light and scanning electron microscopy after *in vitro* incubation in different media and in oesophagus.

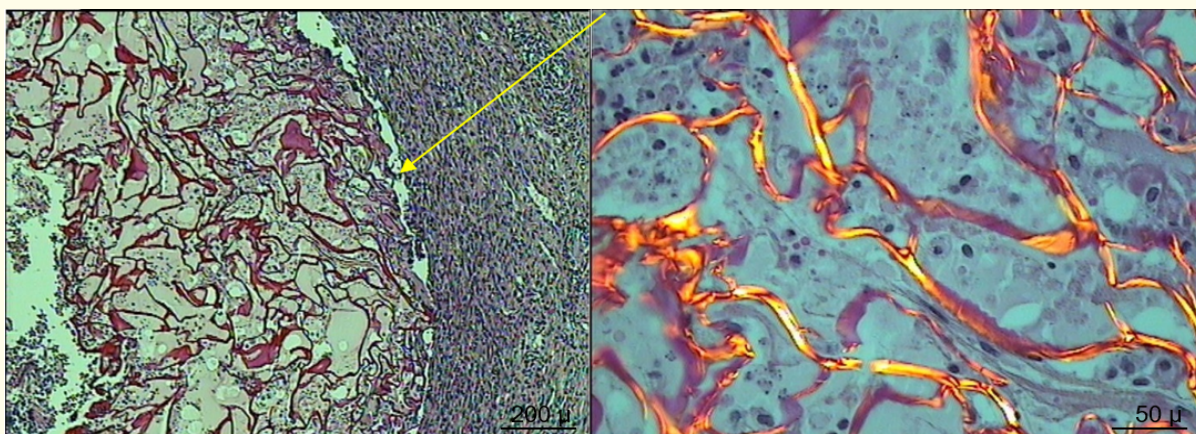
A: Chitosan porous membranes degradation in saline at pH 6. *a.* after 1 hour (Haematoxylin eosin, G x 5), *b.* after 4 hours (H E x 5), *c.* after 24 hours (Hematoxylin eosin, G x 5);

B: Non-porous chitosan tube degradation in saliva (HE and polarized light, G x 5 and x 100).

C: Chitosan tube degradation in the oesophagus (4 days).



A



B



C

Figure 4: *In vivo* biocompatibility and degradation of chitosan.

A: Day 60 after subcutaneous implantation of chitosan membrane and foetal oesophagus into an ear pavilion pouch of mice: mild inflammatory reaction surrounding an excellent growth of the foetal oesophagus (1) and gastric implant (2), terminal stage of chitosan degradation (arrow). Haematoxylin eosin, G x10.

B: Gap between chitosan membrane and surrounding tissues one month after implantation in mice with cell starting to penetrate between the chitosan elements (Haematoxylin eosin, G x 10 and polarized light, G x 40).

C: Roentgen imaging of a chitosan tube two months after their subcutaneously implantation into the anterior part of the neck (arrow).

Control series with insertion of chitosan tube into the intact esophagus did not lead to any complication but the tube could not be found after 2 - 3 days. Addition of sodium bicarbonate (4 g/l) up to pH 7.6 to the drink water allowed the tube to remain present though deformed at day 9 - 10.

Control series with polyethylene and paraffin films have shown that a fibrous capsule formed around the foreign material. The last remained separated from the surrounding tissues and the foetal implant during the whole observational period.

Part II

At the beginning of the experimentation, 7 animals died either during the intervention or in the first hours after it. It was mainly due to vagal shock, anaesthesia drug overdose and accidental excessive blood loss or other technical difficulties preventing the surgery to be completed. Correction of these technical defects, particularly vagal shock by systematic subcutaneous injection of Atropine sulfate 1% after anaesthesia induction, allowed to avoid most of early complications. They were not taken into account in the presentation of the results.

The overall results of the experimental series of Part II are shown in table 3.

Series	Animals Number total / >7 days survival	Complications early/late	Body Weight max loss (%)	Recovery (days)
Chitosan tube alone (oval and circular defects)	41 (18*+23)/20	6/3	14.1 30.3	21 60 - 90
Chitosan tube + GFEI (a. oval, b. circular defects) c. circ.def + MF	35 (6+24+5)/21	a. 0/0 b. 4/0 c. 0/0	a. 13.2 b. 17.5 c. 17.7	a. 14 b. 30 c. 35 - 65
Control (GFEI only)	6/2	6/0	NA**	
Control: circular defect suture	10/0	10/0	NA**	
Intact control	6	0/0	11.7	30 - 60
Total	98	21/3		

Table 3: Overall results of the experiments in part II.

*: In 7 cases death at day 1 was due to technical causes;

** : NA: Not applicable (too short survival delay).

Both control series with esophagus defect repair by simple suture or by fetal esophagus cyst flap ended with a neck abscess within the first 3 - 5 days post-surgery. These series were stopped for ethical considerations.

Series with oval esophagus defect repair using chitosan prosthesis of appropriated diameter (no less than 2 mm) and several stitches fixation gave satisfying results independently of the presence of a FOC flap. The use of too tiny chitosan tube-scaffold (< 2 mm diameter) led to leakage problems and was abandoned (7 cases, see table 3). The BW loss after operation reached 14.1% of the initial BW (11.7% in intact control, 13.2% when FOF was used). The recovery was complete within 30 days (Table 3, Figure 5). The restoration of the 3 layers of the oesophagus wall within 3 months was confirmed by necropsy observations and histologic investigations. Up to 6 months post-surgery a depression of the inner surface of the oesophagus could still be observed at the repair site.

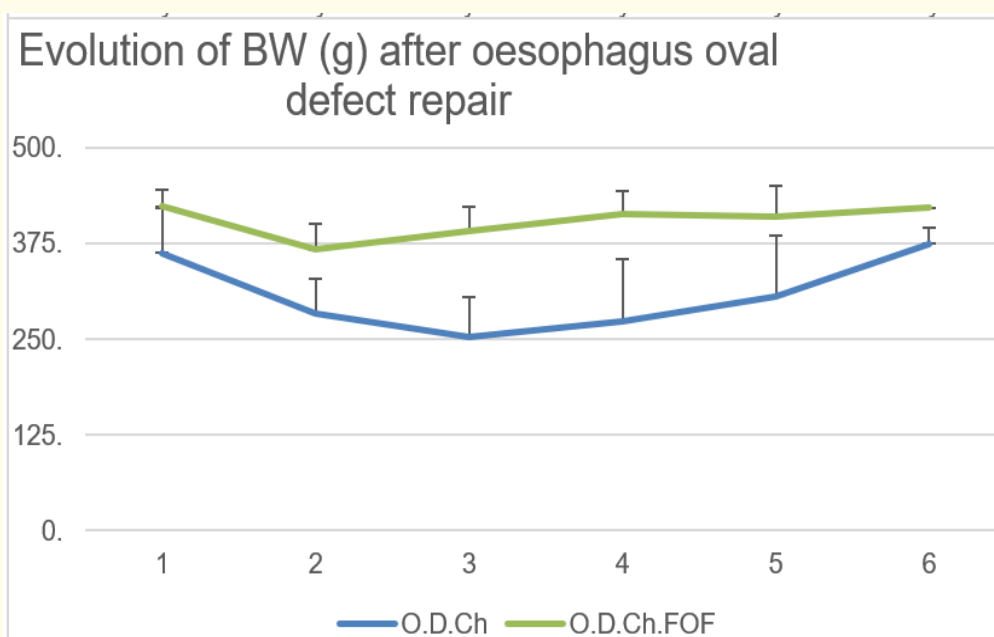
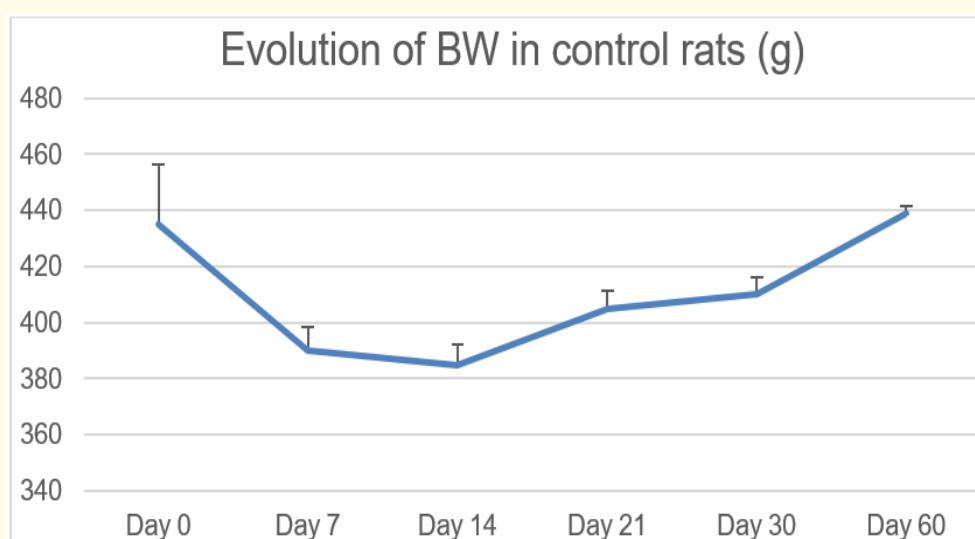
In the series with circular/segmental defect some difficulties were encountered. The main complications observed and the correction/prevention proposed are summarized in table 4.

As a result of these corrective actions, two groups of 20 and 21 animals with respectively chitosan prosthesis alone and chitosan prosthesis combined with grown foetal oesophagus or muscle flap, could be observed till 13 months after surgery.

During the first 2 weeks post-surgery, the BW loss was important with a maximum after 2 weeks (Figure 5). In control animals it reached 11.7%. In circular defect series when chitosan tube was used alone, it reached 30.3%, whereas it did not prevail 17.7% in the other ones (FOF and MF). In these series the recovery delay was also (See table 3). After 1 - 2 months the animals were close to their initial weight (See figure 5).

Nature of the complication	Corrective action
Cardio-respiratory arrest (due to N. recurrens excitation or damage)	Atropine 1% 0.1 ml/150 g BW, subcutaneous, at induction of general anaesthesia
Dehydration	10 ml saline subcutaneous at Day 1 and 2 in spite of free drinking possibility
Excessive weight loss (>10% of initial bw)	Condensed milk with sugar and saline enriched with vitamins and amino acids from Day 3 - 4 till Day 14 - 21
Bezoar or food compaction	Normal alimentation only after day 14 (depending on general condition, additive nutriments maintained). Cages with grids, no contact with straw or sawdust during 1 - 2 month.
Haemorrhage	Pre-operative accurate hemostasis and post-operative drainage
Neck abscess (leakage)	Adequate prosthesis diameter and fixation

Table 4: Main complications and corrective actions in the series with circular (segmental) defects.



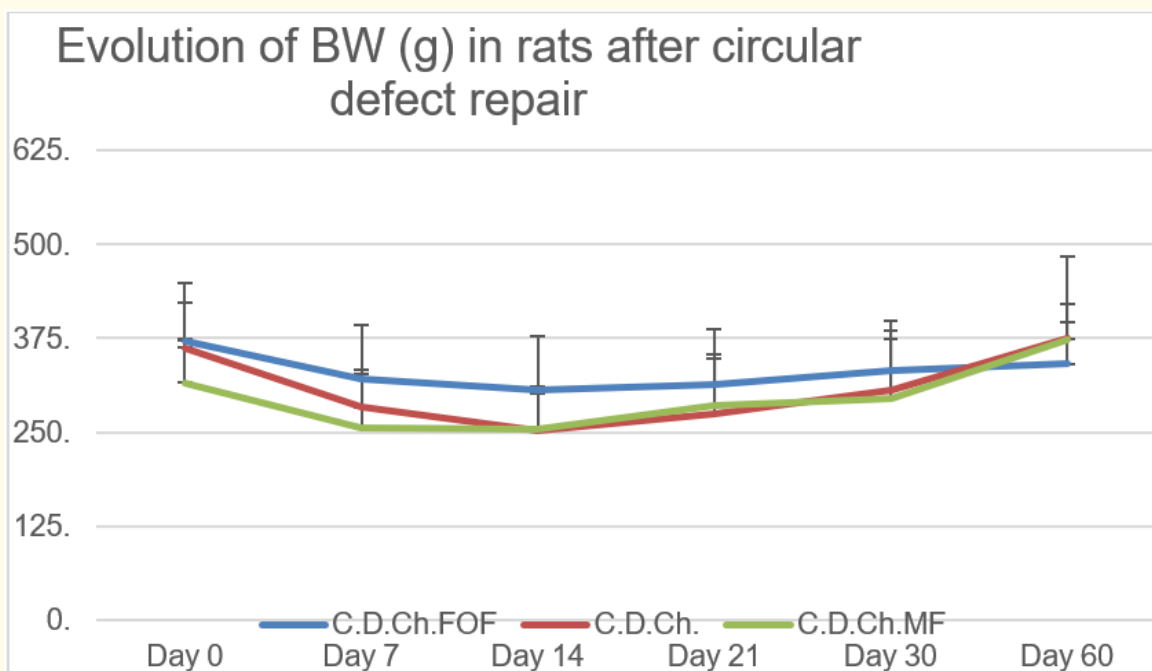


Figure 5: Influence of the different surgeries on the animal BW recovery.

Radiological investigation at late delays has shown a good transit of the Gastrografin® bolus and no significant stenosis or dilatation of the repaired segment (Figure 6).

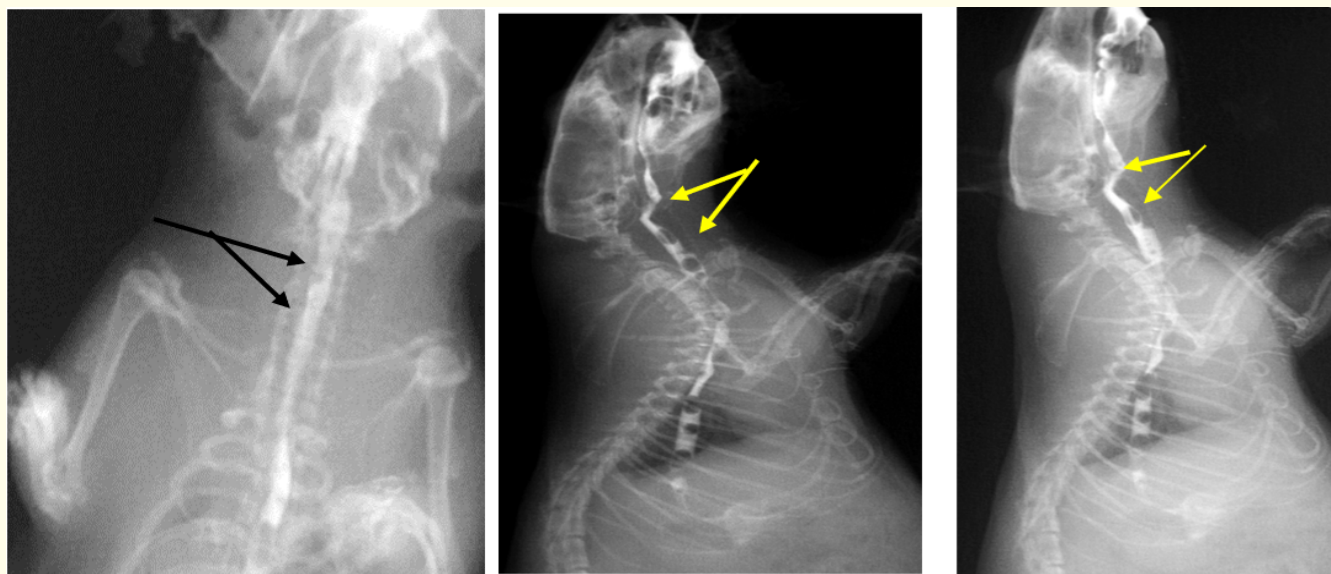


Figure 6: Radiologic imaging of the permeability of the repaired cervical oesophagus (arrows): no significant stenosis (< 10%), good peristalsis.

This was confirmed by necropsy observations in late delay: only in a few cases a difference of less than 10% of the circumference of the repaired segment compared to intact ones was measured (Figure 7).

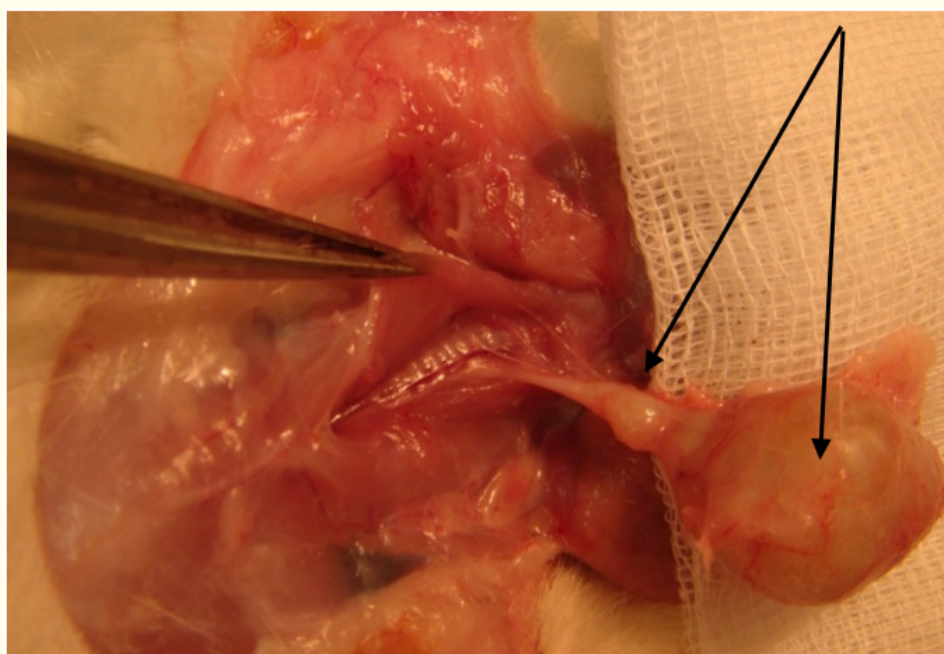
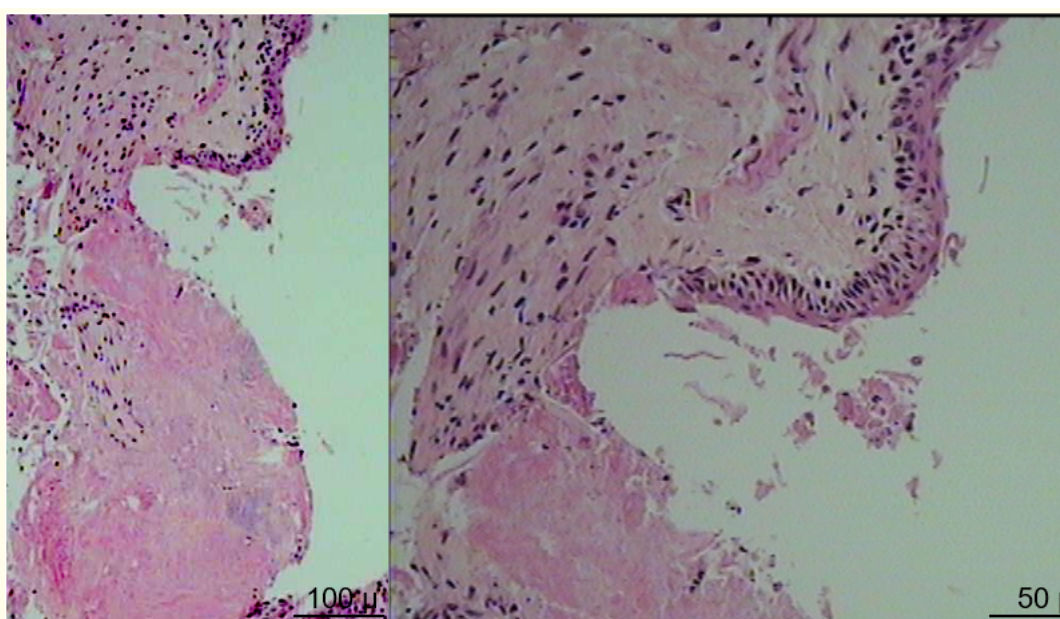


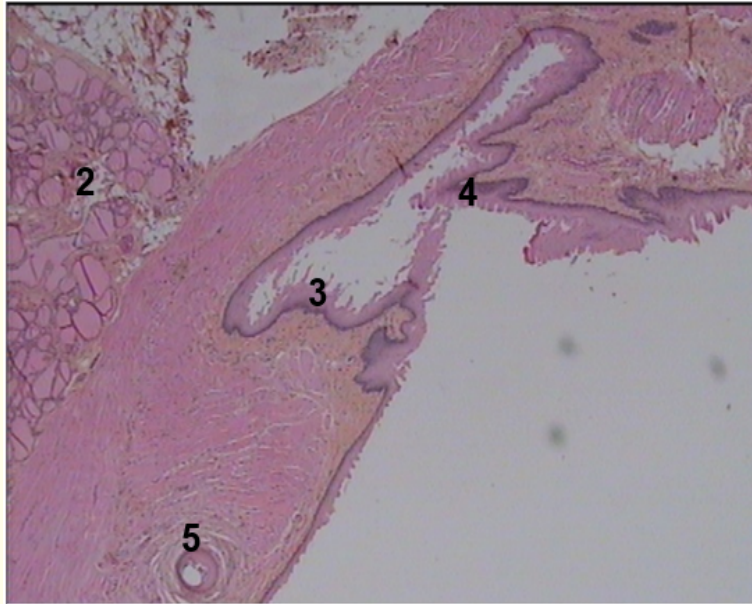
Figure 7: Macroscopic observation of oesophagus defect repair.

Post euthanasia preparation of a repaired cervical oesophagus (arrows) with a remaining link between the cyst and the flap used for repairing the oesophagus segmental defect.

Histologically, at day 7 - 9, a thin fibrous capsule already surrounded the remnants of the chitosan scaffold. At days 14 - 16, the growth of epithelium from the edges of the resection was observed (Figure 8A). Later a muscular layer was observed, very thin at the beginning, then practically of normal size after 4 - 6 months (Figure 8B). Restoration of the three layers, i.e. mucosal, sub-mucosal and muscular layers, of the repaired oesophagus was completed at day 60 but the muscular layer remained thin. After 8 months, it was difficult to distinguish the intact from the repaired organ segment (Figure 8C). Neither late fibrosis nor ulceration was observed up to 11 - 13 months post-surgery. In some cases a slight enlargement or a small depression of the repaired zone was observed that corresponded to a thin muscular layer. The presence of intramural neurons confirmed the complete oesophagus wall restoration and its functional capacity.



A



B

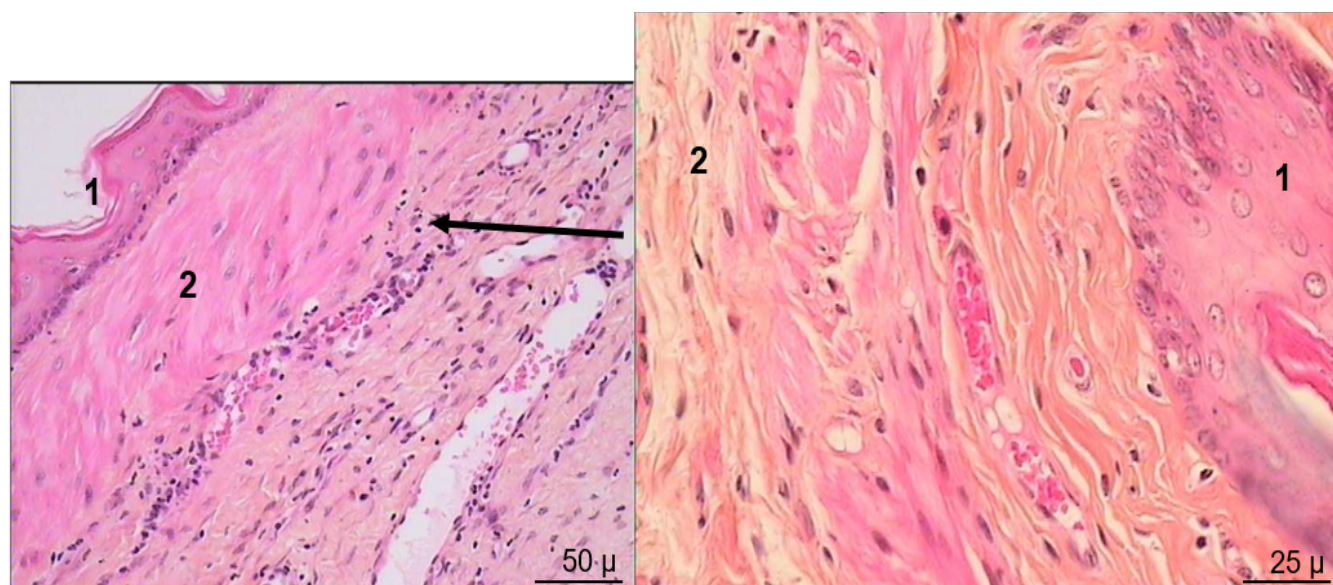


Figure 8: Histology of oesophagus defect repair (Hematoxylin-eosin-saffron).

A: Day 9: Thin fibrous capsule and start of epithelium growth from the edges of cut oesophagus along the inner surface of the fibrous capsule.

B: Day 28: Newly- formed oesophagus wall, with epithelium and forming muscular layer. Arrow: zone of oesophagus reconstruction (difference of muscle layer thickness). 1. Trachea wall, 2. Thyroid gland, 3. Transition muscular zone, 4. Oesophagus with hyperkeratosis on the right, 5. Suture stitch marking the transition zone between genuine and repaired oesophagus epithelium

C: Day 420: Normalization of the oesophagus wall structure at the site of circular defect replacement by chitosan tube scaffold with a flap of in situ grown foetal oesophagus implant. Arrow: transition zone.

Discussion

The current approach in organ reparative surgery, including oesophagus, trachea and heart, is to cultivate stem cells (obtained by different ways) *in vitro*, differentiate them into adult-like cells and tissue and further implant them into the organism using a degradable or non-degradable scaffold [32-35,41,42]. But this strategy remains complex.

We used a different approach to avoid the *in vitro* culture phase [30,31,40,43-45]. Implanting syngeneic foetal oesophagus (stomach, intestine) into the neck site of an adult animal allows the formation and development of a cyst with the same wall structure as the corresponding adult organ. A well-vascularized flap of this cyst was used to reinforce the chitosan tube, bridging the gap (oval or circular) of the oesophagus. The results were encouraging when scaffold properties, surgical technique and follow-up management were adequate.

In our experiments we demonstrated that chitosan tubes are adequate as scaffold for oesophagus repair. Degradation rate of the chitosan tube is to be optimal in order to allow the thin fibrin/fibrous membrane, necessary for a waterproof oesophagus to form. Premature degradation could lead to leakages while late degradation could cause oesophageal wall damage. Slightly alkaline drink water supply during the first 2 post operation weeks has shown useful.

It was also possible to use the capacity of chitosan to form a tight junction with the oesophageal mucosa layer in order to fix the tube into the organ lumen by isolated stiches, avoiding circular ligature which causes cut edges necrosis and slows down wall regeneration. Therefore a correct tube diameter had to be selected.

In order to preserve the development of the new waterproof oesophagus, the rats were fasted during 4 days, receiving only alkalized water (for preventing the quick chitosan degradation), and further only semi-liquid food during 2 weeks. Condensed milk with sugar has proved to be useful for both mechanic protection of the forming wall and caloric providing. Nevertheless the decrease of BW during this period was significant, even in control intact group, and up to 30% in circular defect repair without bio reinforcement. Restoration of the usual diet, while condensed sugar milk was maintained, has enhanced BW recovery through 1 or 2 months after surgery depending on the type of defect (See table 3 and figure 5). Keeping the animals on grids was also a factor of avoiding post-operation complications due to litter ingestion.

The liability of the proposed technique is confirmed by comparing its results with those of the control series. The last have shown that either simple suture of the defect edges or use of a cyst flap alone to bridge the defects, led to significantly higher morbidity and lethality.

In oval defects, chitosan tube alone for temporary bridging the oesophagus, is as safe as a combination with a cyst flap, although in the last case the recovery was faster and complete.

In bridging circular/segmental defects combined chitosan tube with grown foetal oesophagus flap shows significantly better results than chitosan tube alone: less complications, less body weight loss during the first weeks post-surgery, better general condition recovery (See figure 5), and better histological issue. In this series, the use of the optimized technique allowed a 95% survival rate with almost no early or late complications. That may be explained by the conversion of circular defect into a patch one.

Diverticula formation was not found but this risk cannot be fully avoided, especially in case of non-appropriated food ingestion. Stenosis was not significant and not systematic on anatomic pieces (10% in perimeter). Oesophagus function was not disturbed in these animals whose BW - after initial decrease - increased in conformity with standard curves depending on age and sex. (See deglutition investigation by Roentgen serial imaging technique with contrast, figure 6) [40].

It is usually recognized that stenosis in oesophagus reconstructive surgery is due to a partial restoration of the mucosa. Therefore, restoring the oesophagus epithelium is the first aim in oesophagus reconstruction experiments [22]. Nevertheless our results with the use of a FOF or MF flap how the importance of the muscular layer restoration for both prevention of stenosis and a satisfying transit function of the repaired organ.

The results also depend on the length of the bridged gap. In our experiments the length of cervical vertebral column of the adult rat was 18 - 20 mm, the length of the cervical oesophagus varied from 12 to 15 mm, so resection of a segment from 3 to 5 mm long represents about one third of the organ length. Extrapolated to humans, it corresponds to a 5 cm segment. That represents a significantly more important surface to be covered by regenerating tissues and may worsen the human oesophagus repair conditions.

The advantage of the proposed operation: relatively simple and low cost technique applicable to cervical oesophagus circular defects bridging.

The main limits of this technique, if applied to human, (1) the use of FOC flap including two step operation, problem of tolerance between graft and recipient, (2) ethic problem of collecting foetal material. The use of a local muscle flap constitutes a promising alternative.

Conclusion

1. A new combined bio prosthesis for oesophagus defect repair has been proposed and elaborated.
2. Chitosan is a suitable scaffolding material for the oesophagus defect repair because of its controllable degradation.
3. Oval defects are easier to repair than circular ones. In case of oval defect, the use of chitosan tube alone for closing the defect during a few days is enough to obtain a favorable outcome, i.e. a quick and satisfying restoration of the oesophagus wall.
4. Segmental defects may be repaired without early or late complication with chitosan tubes as scaffold combined with a well vascularized muscular flap as reinforcement (conversion of segmental defect into an oval one).
5. The factors of success seem to be: a. tight adaptation of the tubular chitosan scaffold to the oesophagus wall, that prevents the use of circular ligature for waterproofing of the junction; b. reinforcement of the gap by a well vascularized flap (especially when the flap is a part of a cyst formed by the grown foetal oesophagus implant; a muscular flap may also be used); c. optimal conditions for repair of the mucosa and submucosa layers from the cut oesophagus edges and from the FOC flap.

- For stenosis prevention of the newly formed oesophagus segment, the repair of the muscular layer seems to be as important as the repair of the mucosa.

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Conflict of Interest

Any financial interest or any conflict of interest exist.

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