

## Biosynthetic cellulose induces the formation of a *neoduramater* following pre-natal correction of meningocele in fetal sheep<sup>1</sup>

A celulose biossintética induz a formação de uma *neoduramáter* na correção antenatal da meningomielocle em fetos de ovelhas

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### ABSTRACT

**Purpose:** The aim of this study was to compare the effectiveness of two dura-mater substitutes, namely human acellular dermal matrix (HADM) and biosynthetic cellulose (BC), in repairing, in utero, surgically-induced meningocele (MMC) in fetal sheep. **Methods:** A neural tube defect was created at 74-77 days gestation in 36 fetal sheep. They were divided into 3 groups, the control group that did not receive pre-natal corrective surgery, and the other two groups that received corrective surgery using HADM (Group A) or BC (Group B). Both materials were used as a dura-mater substitutes between the neural tissue and the sutured skin. Correction was performed at gestation day 100 and the fetuses were maintained in utero until term. Sheep were sacrificed on gestation day 140. The fetal spine was submitted to macro and microscopic analysis. At microscopy, adherence of the material to the skin and neural tissue was analyzed. **Results:** In the initial phase (pilot), experimentally-induced MMC was performed on 11 fetuses and 4 survived (37%). In the second phase (study), 25 fetuses received surgery and 17 survived (68%). In the study group, 6 fetuses did not undergo repair (control group), 11 cases were submitted to corrective surgery (experimental group) and one fetal loss occurred. Of the surviving cases in the experimental group, 4 constituted Group A and 6 in Group B. Macroscopically, skin and underlying tissues were easily displaced from the BC in all cases it was used; in contrast, HADM adhered to these tissues. To compare the adherence, 4 cases from Group A and 4 in Group B were studied. We observed adherence, host cell migration and vessel proliferation into the HADM all sections from Group A and this aspect was not present in any cases in Group B ( $p < 0.05$ ). In Group B, we also observed that a new fibroblast layer formed around the BC thus protecting the medulla and constituting a “neoduramater”. **Conclusion:** The use of BC seems to be more adequate as a dura-mater substitute to cover the damaged neural tissue than HADM. It seems promising for use in the in utero correction of MMC because it does not adhere to neural tissue of superficial and deep layers (“tethered spinal cord”). Thus, BC minimizes the mechanical and chemical intrauterine damage to the spinal medulla.

**Key words:** Fetus. Meningocele. Spinal dysraphism. Prenatal care. Cellulose. Animal experimentation. Sheep.

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### RESUMO

**Objetivo:** Estudar os efeitos do emprego de dois materiais consideravelmente diferentes quanto à origem e custo na correção intra-uterina da meningomielocle criada experimentalmente em feto de ovino. **Métodos:** Em 36 fetos de ovinos foi criado um defeito aberto de tubo neural, com 75 de dias de gestação. Os casos foram divididos em três grupos: o controle onde o defeito não foi corrigido, grupo corrigido A onde o material utilizado para cobrir a medula exposta foi a matriz dérmica humana acelular (MDHA) e o grupo corrigido B onde o material foi a celulose biossintética (CB). Após a correção realizada com 100 dias, os fetos eram mantidos intra-útero até o termo da gestação. Os sacrifícios foram realizados com 140 dias e a coluna fetal era submetida à análise macro e microscópica

onde foi observada a aderência dos materiais à pele, medula ou tecido nervoso remanescente. **Resultados:** Na fase inicial (piloto), 11 fetos foram operados e 4 sobreviveram (37%). Na segunda fase (estudo) 25 fetos foram operados e 17 sobreviveram (68%). No grupo de estudo, 6 fetos não foram submetidos à correção (grupo controle), 11 casos foram corrigidos e ocorreu 1 perda fetal. Do total de 10 casos, 4 constituíram o grupo A e 6, o grupo B. À macroscopia observou-se deslizamento da pele e tecidos subjacentes sobre a CB em todos os casos onde ela foi empregada e isto não ocorreu em nenhum dos casos onde a MDHA foi utilizada. Para comparar a aderência, foram considerados 4 casos do grupo A e 4 do grupo B. A aderência, caracterizada pela migração de células do hospedeiro e proliferação de vasos para dentro da MDHA, foi observada em 100% dos casos do grupo A e em nenhum caso no grupo B ( $p < 0,05$ ). No grupo B observou-se formação de uma camada de fibroblastos ao redor do material, protegendo a medula, caracterizando a formação de uma “neoduramater”. **Conclusão:** A utilização da película de celulose biossintética parece ser mais adequada como substituto de dura-máter para cobertura e proteção do tecido nervoso que a matriz dérmica humana acelular. Ela parece promissora na correção intra-uterina da meningomielocoele, evitando a aderência do tecido nervoso aos planos superficiais (“medula presa”) minimizando os efeitos deletérios do ambiente intra-uterino sobre a medula espinhal.

**Descritores:** Feto. Meningomielocoele. Disrafismo espinhal. Cuidado pré-natal. Celulose. Experimentação animal. Ovinos.

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## Introduction

Spina bifida is a developmental birth defect that is characterized by the failure of the embryonic neural tube to fully close resulting in malformed vertebrae that do completely envelop the spinal cord.<sup>1</sup> One type of this defect is myelomeningocele (MMC) which has a high incidence in humans (1 to 3 in every 1000 live births)<sup>2</sup>. MMC can be repaired surgically after birth although the success rate varies possibly because of irreparable damage to the spinal cord that occurs pre-natally. Experimental studies suggest that neural tissue may be injured by exposure to chemical (amniotic fluid) and mechanical factors in utero<sup>3,4</sup>. Thus, despite post-natal corrective surgery, a high degree of sequelae can persist, including motor deficiency, urinary and fecal incontinence, cerebellar herniation leading to a hydrocephalus and mental retardation<sup>5</sup>. Furthermore, neurosurgery at birth can not correct the “tethered cord syndrome” caused by adhesion between neural tissue and skin<sup>6</sup>. Since post-natal surgery does not reverse previous neurological damage in utero, operating pre-natally may be more effective. In support of this, studies have shown that in utero repair can prevent or reverse the Arnold-Chiari malformation and hydrocephalus, minimizing post-natal sequelae<sup>7-10</sup>. Several materials have been used as dura-mater substitutes in MMC corrective surgeries that have different origins, manufacture, costs and handling. One such material is the human acelular dermal matrix (HADM) which is a regenerative tissue matrix that has been used in craniotomies and adult spinal cord lesions<sup>11</sup>. It has also been applied as a visceral protection in cases of giant newborn omphalocele<sup>12</sup>. HADM is considered a good alternative if an allograft is not available and it is as pliable as a dura-mater patch intra-operatively. It does not induce adhesions or rejections, although fluid leakage through the material occurred in a few cases<sup>11</sup>. Paek et al. (2000)<sup>13</sup> studied the effects of HADM in ovine models, applied above

MMC as a patch and sutured to the skin. This author observed wound contraction and skin growth over the HADM, preventing posterior herniation of the cerebellum, compared to uncovered controls. Another material experimental dura-mater substitute is biosynthetic cellulose (BC)<sup>14,15</sup>. This Brazilian manufactured membrane is a low-cost material that has been used in plastic surgery as a temporary skin substitute for second degree burns. BC has been used during corrective surgery to cover the exposed spinal cord in a fetal rabbit model and showed no signs of rejection<sup>16</sup>. The aim of this study is to compare the effectiveness of HADM and BC in protecting neural tissue after in utero surgery to correct experimentally-induced MMC in a fetal ovine model. Sheep were chosen for the experimental animal since they are used widely in experimental studies of gestation and fetal surgery, mainly because both its size and anatomy are similar to the human fetus. Furthermore, sheep can be easily handled in pen, have a small number of fetuses (1 or 2) and a low rate of premature labor<sup>1</sup>.

## Methods

Thirty three mixed breed Hampshire Down sheep were obtained from a single breeder. Pregnancy was dated from intra-cervical insemination, during the natural estrous (twice a year). Three of them were twin pregnancies, so a total of 36 fetuses were studied. The study was approved by Dante Pazzanese Institute Ethics Committee. The animals were transported from farm and given a minimum adaptation period of 4 d. They were maintained in a semi-open pen with natural day/night variation.

### *Experimentally-induced MMC*

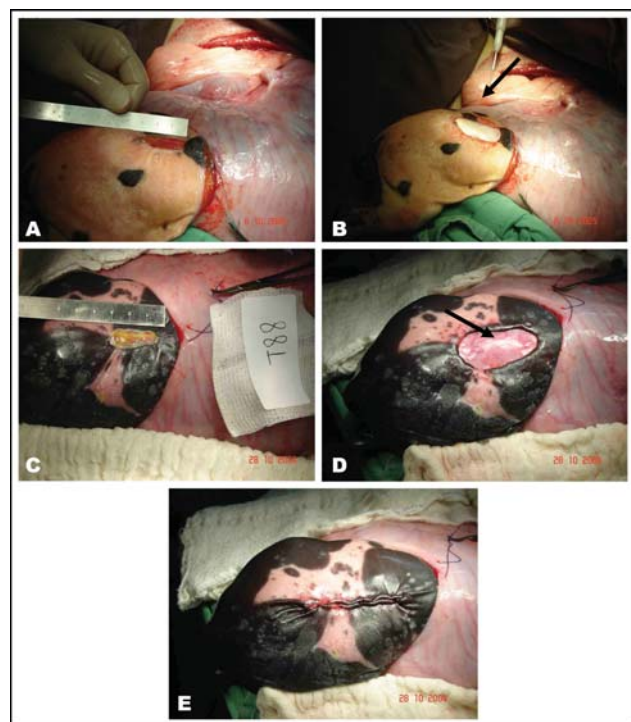
Surgery to induce MMC was performed in all fetuses between 74 and 77 days of gestation. The initial

phase (pilot group) involved 9 animals, pregnant with 11 fetuses; the second phase (study group) consisted of 25 fetuses. In the corrected group, reparative surgery was performed between 93 and 112 days of gestation. These animals remained in utero for the term of gestation. In the control group the MMC was induced but the correction surgery was not performed. Before surgery, animals were submitted to 48-hour food and 24-h fluid fast. The anesthetic protocol consisted of preanesthetic medication while still in the pen (acepromazine 0.2 mg/kg + midazolam – 0.3 mg/kg IV), allowing the animal to be transported while sedated. Hair was removed immediately before entering the operating room. Animals were anesthetized with intravenous thiopental (7.5 mg/kg), followed by orotracheal intubation, and then maintained with 2% halothane. During the operation and post-anesthetic recovery a total of 15 ml/kg/hr of 0.9% saline containing 15 ml/kg/h of 50% glucose. A single dose of the antibiotic prophylaxis enrofloxacin (5 mg/kg) was administered. The animal was positioned in right lateral decubitus, and a left para-mammary laparotomy was used to expose the uterus. A 5 cm hysterotomy was performed just above the fetal spine allowing back paws and tail exteriorization. The amniotic fluid was then removed (100 ml) and maintained in a heated saline bath to posterior restitution during hysterorrhaphy. The skin resection over lumbosacral spine measured 3.0 x 1.5 cm. Bilateral paravertebral muscles resection and a complete laminectomy of four lumbar vertebrae extensions were performed. Dura-mater was incised with a scalpel, cerebral spinal fluid (CSF) leakage was visualized and the medullar spine was incised to the central canal. The medulla was then left exposed. The fetus was returned to the uterus which was then closed, afterwards by the abdominal maternal wall was sutured in layers. Fetal heart-rate was checked through a transoperative ultrasonography (US) and animals were returned to the pen after spontaneous breathing started. Utero-lytics were not used during or after the procedures.

#### *Corrective surgery for MMC*

The corrected cases were divided in two groups according to the material used to cover the neural tissue: Group A received HADM (AlloDerm<sup>®</sup>, Lifecell, USA) and Group B was given BC (Nexfill<sup>®</sup>, Brazil; Dermafill<sup>®</sup>, USA). The same perioperative conditions used for the defect creation were repeated for the corrective procedure up to the point of fetal back exposure. Both materials were applied over the damaged neural tissue according to the previously developed technique used in the fetal rabbit model<sup>16</sup>. Briefly, we under cut 1.0 cm of the skin beyond the margins of the defect and placed either HADM or BC over the exposed neural tissue and under the skin edges; skin was then approximated to cover all the material (Figure 1). HADM is obtained from

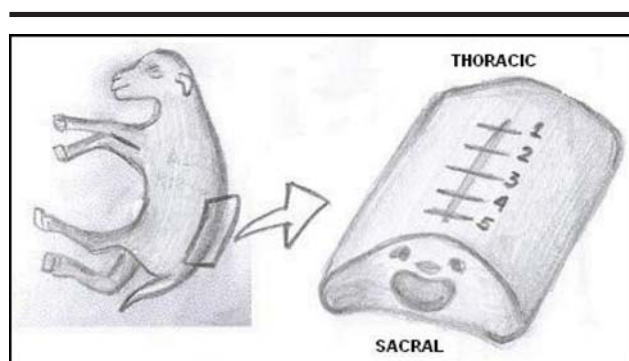
human cadaveric skin supplied from tissue banks, processed for epidermal removal with a high ionic-strength solution and dermal separation. Subsequently, cells are removed and the remaining bioactive components and extra cellular matrix (collagen and elastin) are preserved. The result is a foundation for normal revascularization, cell repopulation and tissue remodeling, becoming the patient's own tissue. It is preserved by freeze-drying, needing special storage and lower temperatures for transportation. The manufacturing process is complex and costly<sup>17,18</sup>. The BC film is produced by *Acinetobacter* bacteria fermentation using specific technique developed in Brazil, approved by the FDA (USA) in 1995. The cellulose mass is sliced, washed and deproteinated using special solvents. The storage and transportation are at room temperature and it can be reesterilized in ethylene oxide. It has a significantly lower cost compared to HADM. It was developed to protect burn areas or to cover graft donor sites, allowing reepithelization under the material. When the new skin is formed, the BC detaches spontaneously<sup>14</sup>.



**FIGURE 1** - Stages of corrective surgery for experimentally-induced MMC in sheep fetus. (A) Exposed fetal back during correction surgery at 100 days of gestation. Note the previously created neural tube defect. (B) Human acellular dermal matrix (HADM; black arrow) covering the defect. (C) Same aspect of another animal at 100 days of gestation, with induced MMC. (D) Biosynthetic cellulose (BC; black arrow) was used to cover the defect before it is recovered with skin. (E) Postoperative sutured skin

### Fetal harvesting and macroscopic analysis

Animals were sacrificed at gestation day 140. The same pre-anesthetic medication was used for maternal-fetal sacrifice, but the thiopental dose was increased to 20 mg/kg to guarantee fetal sedation. After a few minutes, a 19.1% KCl bolus was injected into maternal circulation (0.4 ml/kg). After maternal cardiac arrest, the abdominal wall and uterus were opened. If fetal heart activity was detected a 5 ml KCl bolus was administered to the fetus. The fetuses were photographed, weighed and submitted to macroscopic and microscopic analysis. The entire fetus was fixed in formalin for 7 d, after a sample of the spine was removed. This specimen incorporated one vertebra above and one below the defect region, along with 3 cm lateral margins of surrounding tissues, all layers included. To prepare slides, the specimens were incubated for 4 wks in EDTA buffer solution for decalcification. Hematoxylin-Eosin (HE) and Masson staining were performed in a total of five standard sections, containing the whole defect area in both groups (Figure 2). All the samples were analyzed by the same pathologist (PHS). Statistical analysis was carried out at Epidemiology and Statistical Laboratory of Instituto Dante Pazzanese applying the *Fisher test*, with a 5% significance level.



**FIGURE 2** - Anatomical planes from which the five tissue sections were made for microscopic analysis, containing all areas of the defect

### Microscopical analysis

The adherence to adjacent tissues was analyzed in both superficial (next to skin) and deep (next to neural tissue) material surfaces. The presence of cells and vessels that invaded from adjacent tissues was evaluated.

## Results

### Survival rate and prematurity

The study was divided into two phases: i) pilot and ii) study. The pilot phase was conducted first and

involved standardizing the technique for producing and correcting the MMC defect; a total of 11 fetuses had experimentally-induced MMC. Of this group, 7 fetuses died representing a survival rate of 37% (Table 1). In the study phase, 25 fetuses received MMC and 8 fetal losses occurred. Thus, the survival rate, after the creation of the defect, was 68%. Among the 17 surviving fetuses, 6 did not undergo corrective surgery (control group) and 11 received the reparative operation (corrected group). Of the former group, 2 had premature deliveries; of the corrected cases, 1 fetal loss occurred (9%) and 2 premature deliveries were observed (Table 2).

### Surgically corrected cases

A total of 10 corrected cases were available for macroscopic and microscopic analysis: 4 cases in Group A that received HADM and 6 cases in Group B that received BC. Macroscopically the skin was completely closed in 1 case in the control group, 2 cases in Group A and 5 cases in Group B. Macroscopically, during dissection, the skin and underlying tissues were easily displaced from the BC in all cases in Group B. In contrast, HADM was firmly adhered to adjacent tissues in Group A.

### Microscopical analysis

In the control group, histological analysis revealed that the medulla was typically exposed and in 4 out of 6 cases was destroyed. Even in cases in which the skin was partially or completely enclosing the defect, the medulla under the skin was damaged. It appeared that the greater the “in utero” exposure, the greater the damage to the neural tissue. Four weeks after the defect creation, erosion extended to the posterior horns; the medullar central canal and anterior horns were preserved (“flat medulla”). If the fetus was remained in utero for 8 weeks, a typical MMC-like defect (total destructive lesion of medulla) was observed. In the corrected group, for comparison purposes, only cases that stayed in utero a minimum of 28 days after correction were used; thus, a total of 4 cases were analyzed in Group A and 4 in Group B. In Group A, all 4 cases showed blood vessel ingrowth from the host tissue to the HADM and the implant was integrated to the skin with no cleavage plane between the animal dermis and the HADM. The same aspect was found where the HADM contacted the medulla.

**TABLE 1** - Gestational age at the time of surgical procedures and follow-up of fetuses that received experimentally-induced MMC and corrective surgery in the pilot study

Pilot	Group	GAC	GACorr	GASacr	Evolution	Macroscopic Analysis
1	Control	77	-	98	PS (1)	Open Def 3,0 x 2,5cm
2	Control	77	-	93	PD	Open Def 3,0 x 2,5cm
3	Control	74	-	107	PD	Open Def 4 x 3,5cm
4	B	75	93	133	PD	Wound healing 70%
5	Miscarriage	73	-	80	IUD	-
6	Miscarriage	73	-	-	MD+IUD (transoperative)	-
7	Miscarriage	72	-	-	2po IUD	-
8	Miscarriage	75	-	-	2po IUD	-
9	Miscarriage	73	-	80	IUD	-
10	B (Miscarriage)	76	92	92	IUD transoperative	-
11	A (Miscarriage)	74	-	107	2po IUD	-

PS – pathological study

GAC: gestational age at defect creation

GACorr: gestational age at correction

GASacr: gestational age at maternal-fetal sacrifice

PD: premature delivery

(1) – sacrificed to PS analysis (first animal)

po – pos-operative day

A – Group using HADM

B – Group using BC

Control – Group unrepaired

IUD – Intra Uterine Death

MD – Maternal Death

**TABLE 2** - Gestational age at the time of surgical procedures and follow-up of fetuses that received experimentally-induced MMC and corrective surgery during the study phase

Cases	Group	GAC	GACorr	GASacr	Evolution	Macroscopic analysis
1	Control	75	-	140	-	Wound healing 100%
2	Control	75	-	139	-	Open Def 4 x 2 cm
3	Control	75	-	139	-	Wound healing 70%
4	Control	75	-	112	PD	Open Def 3 x 2,5 cm
5	Control	75	-	111	PD	Open Def 3 x 2 cm
6	Control	78	-	140	-	Open Def 4 x 1 cm
7	Miscarriage	74	-	76	IUD 2o PO	-
8	Miscarriage	75	-	76	IUD 1o PO	-
9	Miscarriage	75	-	76	IUD	-
10	Miscarriage	75	-	75	IUD	-
11	Miscarriage	74	-	75	IUD	-
12	Miscarriage	77	-	90	IUD	-
13	Miscarriage	77	-	85	IUD	-
14	Miscarriage	77	-	90	IUD	-
15	A	75	105	138	-	Wound healing 100%
16	A	75	105	137	-	Wound healing 100%
17	A	77	105	134	-	Wound healing 70%
18	A	77	105	138	-	Wound healing 50%
19	B	75	105	105	IUD in correction day	-
20	B	75	103	140	-	Wound healing 100%
21	B	75	103	118	PD	Wound healing 70%
22	B	74	103	118	PD	Wound healing 100%
23	B	75	97	138	-	Wound healing 100%
24	B	75	103	137	-	Wound healing 100%
25	B	75	98	126	PD	Wound healing 100%

GAC: gestational age at defect creation

GACorr: gestational age at correction

GASacr: gestational age at maternal-fetal sacrifice

PD: premature delivery

po – pos-operative day

A – Group using HADM

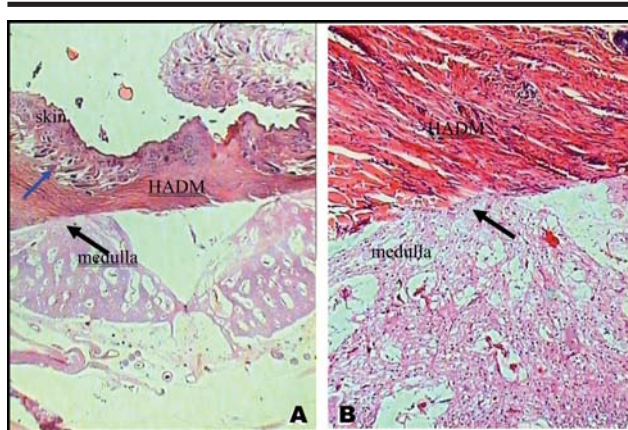
B – Group using BC

Control – Group unrepaired

IUD – Intra Uterine Death

Multiple sites of adhesion to neural tissue, bypassing vessels and cell infiltration were also observed (Figure 3). In Group B that received BC during corrective surgery, all 4 cases showed that the BC was completely occupied by a newly formed layer of fibroblasts (Figure 4). This fibroblast layer was in anatomic continuity with the dural margins that form a new cell layer resembling the dura-mater itself, a “neodura-

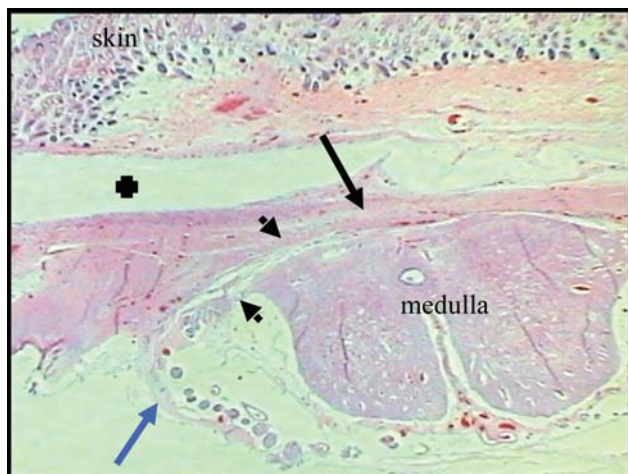
mater” layer (Figure 5). No connective tissue or collagen appeared to have invaded the BC layers. Furthermore, blood vessel proliferation, cell ingrowth and adhesion to surfaces (superficial or deep) were not evident. These characteristics described for Group B were markedly different from those seen in Group A; these group differences were statistically significant ( $p=0.029$ ; Fisher test).



**FIGURE 3** - Fetus in Group A that received corrective surgery for experimentally-induced MMC using human acellular dermal matrix (HADM). (A) HADM adhered to the skin (blue arrow) and to neural tissue (black arrow). Hematoxylin-Eosin stain – 16x. (B) Detailed aspect of the same section shown in panel A. Note the ingrowth of cells from the medulla (black arrow) into the HADM. Hematoxylin-Eosin stain – 100x



**FIGURE 4** - Histological feature of the biosynthetic cellulose (BC; black cross). No ingrowth of cells into the material was observed. Note the fibroblast layer covering the deep and superficial surfaces (black arrows). Hematoxylin-Eosin stain – 50x



**FIGURE 5** - Histological aspects of the “neoduramater” formation. Note the original duramater (blue arrow) in anatomical continuity (arrows heads) with the newly formed fibroblast layer (black arrow) underneath the biosynthetic cellulose (BC; black cross). Hematoxylin-Eosin stain – 16x

### Discussion

The classical MMC corrective neurosurgery performed at birth involves dissecting tissues adjacent to the defect and suturing the dura-mater to protect the medulla. Despite these reparative measures, nerve root scarring at the site of MMC repair happens in 10 to 13% of the cases. This condition can lead to tethered-cord syndrome with neurological-deterioration, back and leg pain, incontinent bladder, spasticity, change in motor or sensory level in lower extremities, severe scoliosis and other occurrences<sup>6,19</sup>. Multiple biological materials, such as collagen, autologous muscular fascia, muscle flaps and acellular human dermis have been used as a dura-mater substitutes for reconstruction or duraplasty<sup>11,13,14</sup>. Intrauterine repair success of correction-prone malformations depends on many factors, such as gestational age, stage of disease in utero, the surgical technique used for repair, surgical time, uterine wall injury, amniotic fluid leakage, dissection and suture extension in fetuses. This study compared the effectiveness of two different types of dura-mater

substitutes, namely HADM and BC, for the repair MMC in fetal sheep using a simplified technique for closure of the defect. An ovine model was chosen because its validity had been previously established<sup>20</sup>. Our study used fetuses with surgically created neural tube defects that were maintained in utero for four weeks to allow the neural tissue injury to occur, simulating the real environment of a human MMC defect. Either HADM or BC was placed over the neural tissue and under the sutured skin to assess their ability to protect the medulla. In the present model, our goal was to protect the medulla from the hostile intra-uterine environment thus avoiding progressive damage and preventing the medulla from adhering to the surrounding tissues which could lead to tethered cord syndrome. We showed that BC was more effective in preventing neural injury and produced less problems related to the corrective surgery than HADM. BC did not adhere to neural tissue, or cause a giant cell reaction or blood vessels to proliferate at the site of injury. Moreover, the fibroblast layer formed an envelope around the BC creating a cleavage plane that would most likely be easier to dissect during post-natal surgery. In contrast, HADM was inundated with multiple blood vessels within its layers, adhered to neural tissue and skin, and showed an ingrowth of cells. In addition to its superiority in avoiding neural injury, BC offered other advantages over HADM. We found that BC was easy to handle at surgery and was ready to use without needing any prior preparation. Each film was 10 x 15 cm, a size large enough to allow tailoring to cover the entire lesion found at surgery. On the contrary, HADM was costly, had to be acquired at a specific size, and required a saline warm bath for 30 minutes before use. Similar findings have been reported both in animals as humans<sup>17,18,21</sup>. For example, HADM used for abdominal wall reconstruction in a rabbit model, showed the expected interaction with the host tissue as illustrated by revascularization and skin ingrowth on the material<sup>21</sup>. From our point of view, HADM is not appropriate for the intra-uterine MMC repair. Our findings are concordant with the observations of Farmer *et al*<sup>6</sup> on the correction of MMC in human fetuses. Using the fetal open surgery approach, these authors used HADM in their first cases and found post-natal corrective surgery to be much more difficult, and therefore abandoned its use in subsequent cases. It was also applied as a patch over MMC fetal ovine models, placed in contact with amniotic fluid and not as an interface between the neural tissue and skin<sup>13</sup>. The authors described wound edge retractions and no “gross” adhesion between cord and patch. However the HADM studies did not involve a comparison to BC and the studies focused on cerebellar herniation and post-natal neurological functions and not on the local effects of these materials on the damaged spinal cord. Two other interface materials (biomatrices) were studied by Eggink *et al*<sup>22</sup>, one obtained from small intestinal submucosa and

the other from bovine tendon collagen. These biomatrices were used for the acute correction of a neural tube defect induced in sheep. They compared both materials with the simple closure of the skin over the defect using suture. They found no differences in postnatal outcome (hydrocephalus, cerebellar herniation or urinary incontinence) among the three groups. As defect creation and its correction occurred during the same procedure, there was no long term exposure of the neural tissue to the amniotic fluid making difficult to compare their findings with ours. Our histological findings for BC-treated animals were also similar to what has been reported in the literature. BC was used as a dura-mater substitute by Mello *et al*<sup>14</sup> in dogs submitted to craniotomies, with encouraging findings. After this, Pedreira *et al* (2003)<sup>16</sup> successfully tested BC in rabbit fetuses for MMC repair. Mello *et al*<sup>14</sup> stated that the BC might be dissolved in tissue alkalis, however, during a maximum 270 day period, they found no evidence of BC digestion or absorption. We believe that this period is enough to keep the exposed neural tissue safely covered throughout gestation. The film detaches from cerebral tissue even after this period, mimicking the dura-mater<sup>14</sup>. These characteristics guarantee the persistence of the BC throughout gestation without modifications. At birth we propose definitive correction by classic neurosurgical techniques. The fibroblast layer that formed under the BC, in continuity with dura-mater, probably will have the expected effect of avoiding fluid leakage, constituting a “neodura-mater”. In our opinion the characteristics and histological findings in our experiment showed that BC avoided the adhesion between the tissues, leading to convenient neural tissue protection during intrauterine period.

### Conclusion

The use of BC appears to be a more effective choice than HADM to for spinal cord protection for the in utero repair of experimental MMC. BC was superior in intra-operative handling and avoiding neural tissue adhesion. This latter feature is an advantage facilitating the removal of BC in the post-natal corrective neurosurgery. Thus, our findings suggest that pre-natal application of BC will protect the medulla from the hostile intra-uterine environment thus avoiding progressive damage and ensuring a greater chance that post-natal surgery will correct MMC.

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