

# An overview of cell sheet transplantation for *in utero* cellular repair of fetal myelomeningocele

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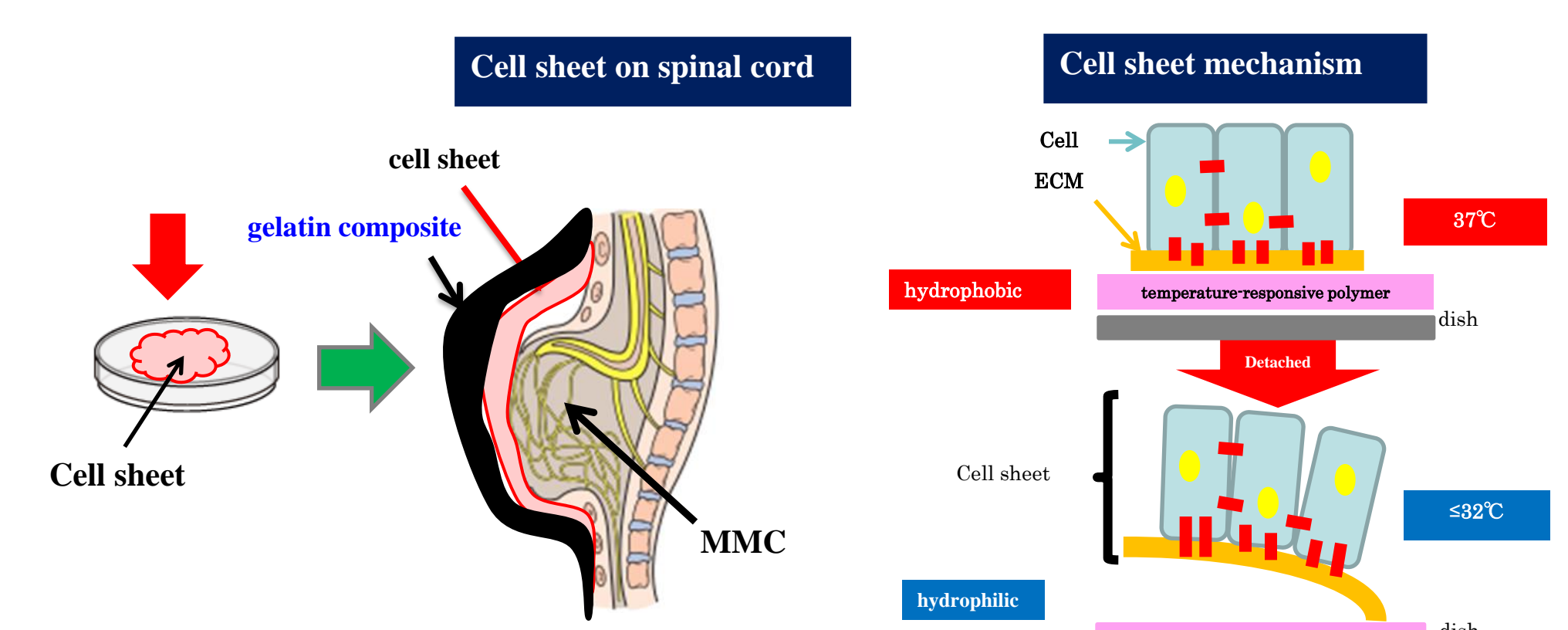
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## Objective

Myelomeningocele (MMC) causes a devastating disability with significant morbidity and mortality within a first few decades of postnatal life. MMC was the first nonlethal disease generally accepted as an indication of fetal surgery and is now the most common anomaly to be corrected by open fetal surgery.

Although current fetal surgical repair has improved clinical outcome rather well, more beneficial approach is now highly expected. We have been working on tissue engineering (cell sheet technology) as a means of restoring damaged neural tissues of MMC *in utero*. We report the current research results of therapeutic feasibility of gelatin composite cell sheet (rat myoblast origin) transplantation along with accompanying problems to be overcome.

### Cell sheet technology (CST):



## Method

Time-dated pregnant Sprague-Dawley rats (E-10 days) were gavaged fed with retinoic acid (60 mg/kg in olive oil) to prepare rat MMC models. A sheet like cultured L6 myoblast cells were prepared using a “thermo responsive dish” which enable a cluster of cells to detach by thermal control of its hydrophobicity. Cell sheet was prepared and transplanted onto the fetal MMC at the age of E-19 days following maternal anesthesia, laparotomy and hysterotomy. Following cell sheet transplantation, the experimental fetal rats stayed inside the uterus (in an amniotic fluid environment) up to 24 hrs. After the animal sacrifice, we evaluated the MMC region that had undergone cell sheet transplantation histochemically.

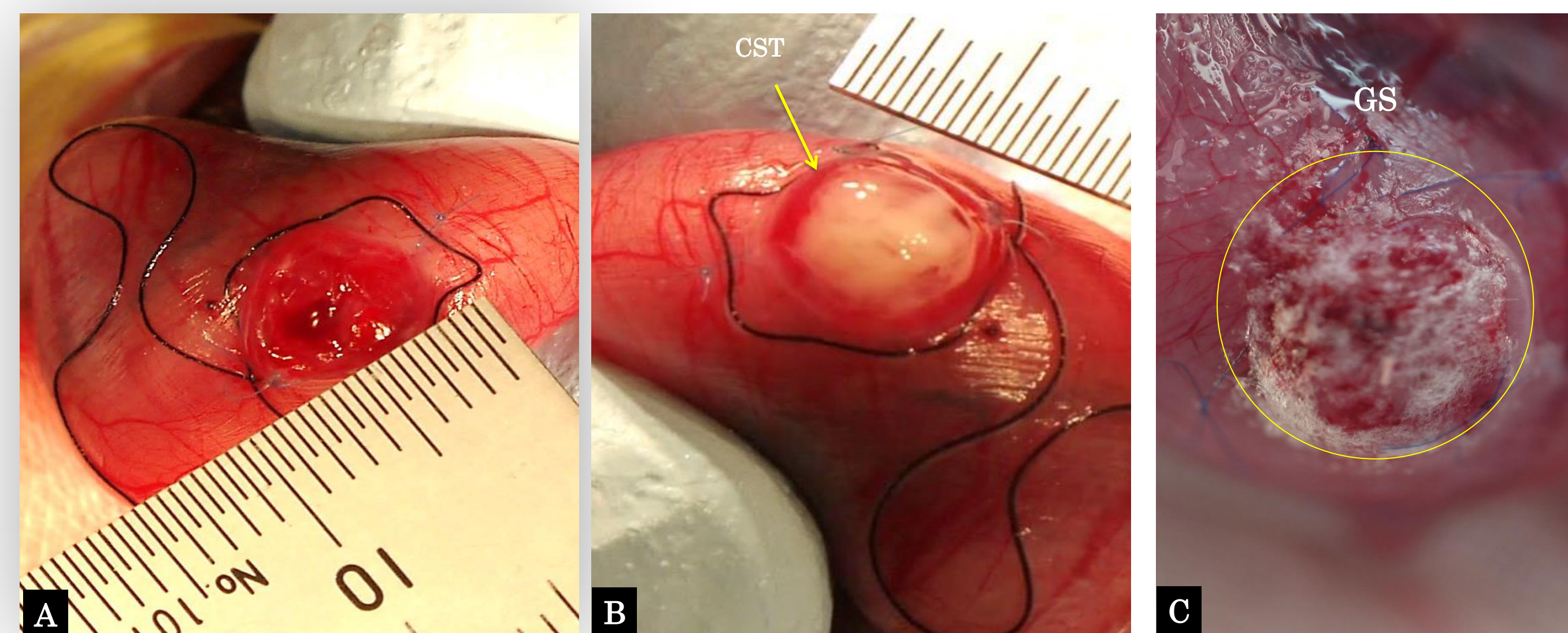
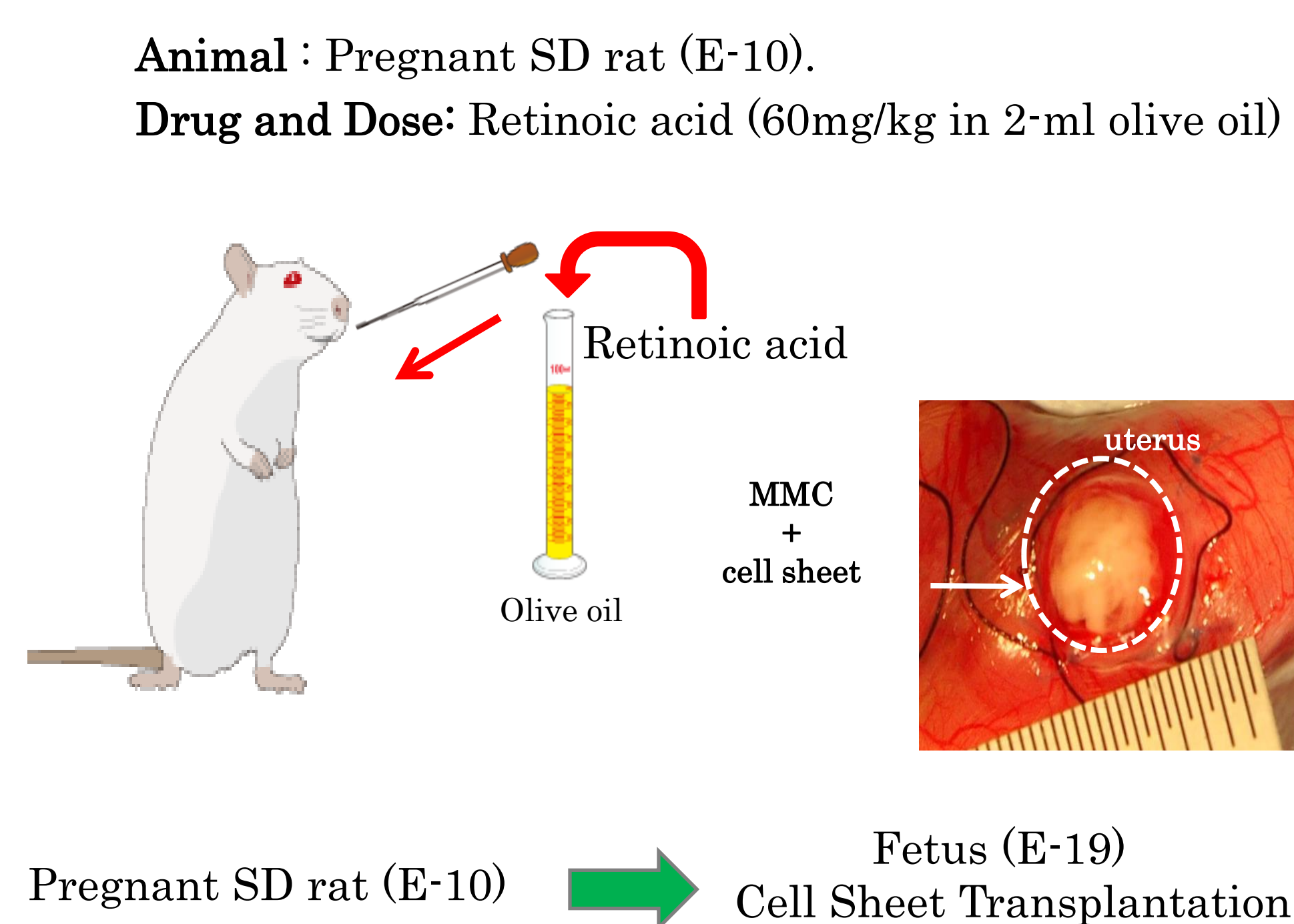


Fig a and b shows the open cell sheet transplantation procedure on the defective area in rat fetus. Fig C shows the gelatin covering of cell sheet transplantation. CST: cell sheet transplantation

## Results

Experimentally, lumbosacral MMC lesions were induced by enforced oral administration of retinoic acid (in olive oil) to pregnant rats at a dose of 60mg/kg/body weight (at E-10 days). High resolution micro CT was used to diagnose those pregnant rats as having fetal MMC at E-19 days. As early as 4, 6, and 24 hrs after transplantation following hysterotomy, the cell sheet attached onto the MMC lesion histochemically demonstrated positive myoblast markers including neural growth factor at the region of cell sheet transplantation. Currently, in place of the cell sheet, using gelatin composite sheet as a new modality which is likely to facilitate the procedure with an improved sheet durability even under a specific underwater environment (in amniotic fluid).

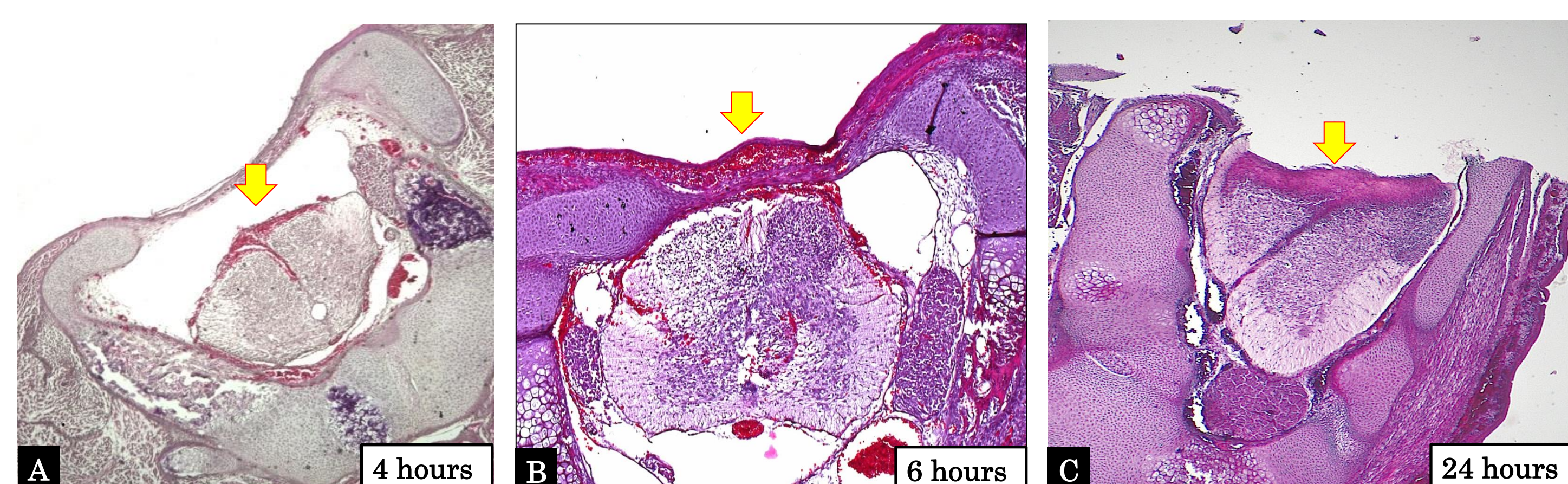


Fig a, b and c shows the H-E staining of 4, 6 and 24 hours of cell sheet transplantation inside the uterus

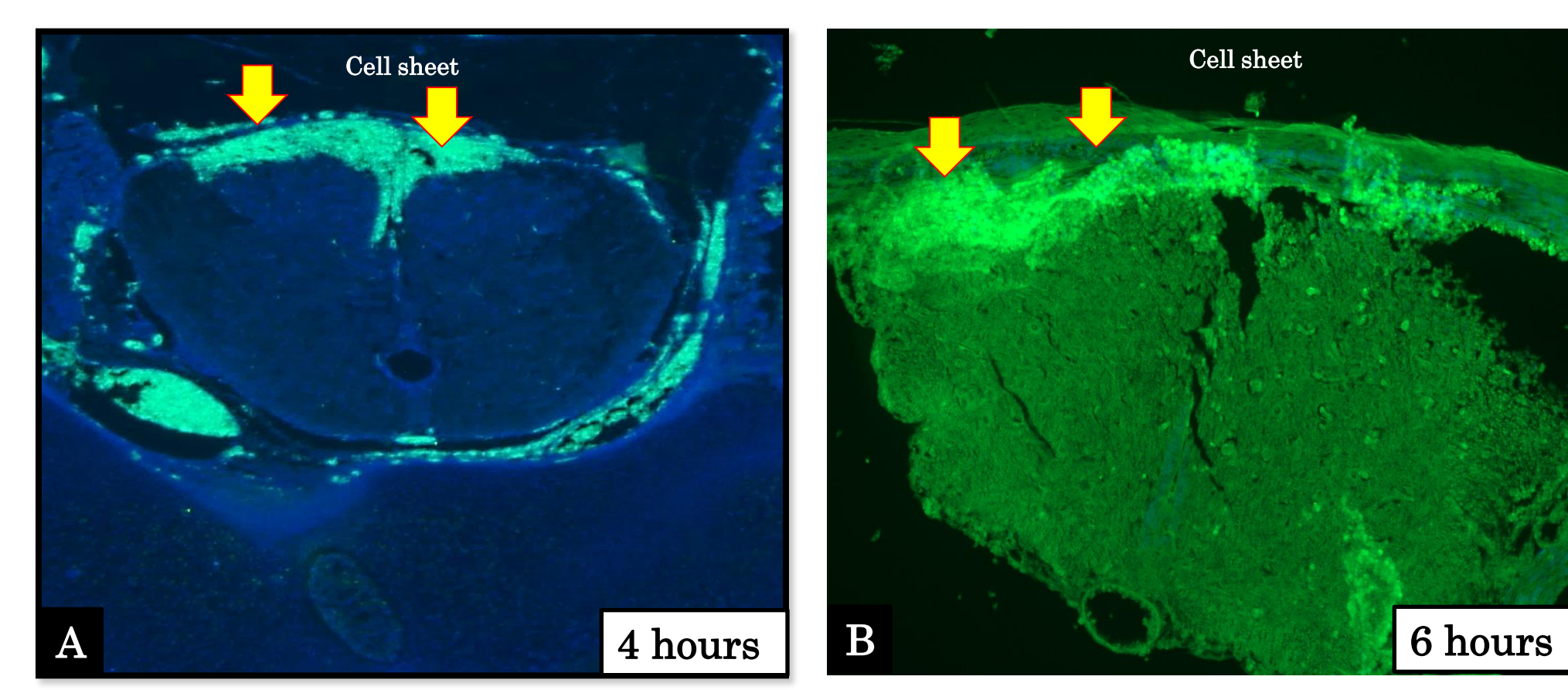


Fig a and b shows the immunostaining of nerve growth factor positive cell sheet transplanted fetus after 4 and 6 hours.

## Conclusion

We report the usefulness of cell sheet technology for fetal myelomeningocele treatment. Gelatin composite sheet worked better as a new modality which improves sheet durability even in the amniotic fluid environment. To know the mechanism and function of the cell sheet attached onto MMC lesion, we need to do further experiments to identify the best timing of intervention.