A Tissue Engineering Approach for Prenatal Closure of Myelomeningocele

Can injectable scaffolds induce tissue regeneration for closure of Myelomeningocele?

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Introduction

Myelomeningocele (MMC) is a common and devastating malformation. As an alternative to fetal surgical repair, tissue engineering has the potential to provide a less invasive approach for tissue coverage applicable at an earlier stage of gestation. We have previously evaluated the use of gelatin hydrogel composites composed of gelatin sponges and sheets as a platform for tissue coverage of the MMC defect in the retinoic acid induced fetal rat model of MMC1. In the current study, we compare our previous composite with gelatin microspheres as a possible injectable scaffold for tissue ingrowth and cellular adhesion within the amniotic fluid environment. We also examine the relative efficacy of various bioactive protein coatings on the adhesion of amniotic fluid cells to the construct within the amniotic cavity.

Materials and Methods

Open surgical technique was utilized on the rat model of Retinoic Acid (RA) induced MMC. At E18 fetal rats were exposed by hysterotomy and underwent application of the gelatin hydrogel composite scaffold on the MMC defect. Survivors were harvested at E22 for evaluation. First, dehydrothermal cross-linked gelatin sheets overlying a either a gelatin sponge or gelatin microspheres were compared for support of epidermal ingrowth. The length of new epidermal ingrowth from the edge of the keratinized epidermis was measured by histologic assessment. Next, amniocyte adherence to either gelatin sponge or gelatin microsphere constructs placed on MMC was evaluated. Viability of adherent cells was evaluated by MTT based colorimetric assay. Finally, cellular adherence was compared among gelatin sheets coated by bioactive proteins (collagen I, IV, fibronectin, laminin) by immunohistochemistry and analysis of cellular proliferation. One way ANOVA or Tamhane post-hoc test were used for data analysis.

Results

Excellent epidermal ingrowth (Fig 1) and cellular adherence (Fig 2) were supported by both gelatin microspheres and sponges. Also, cellular adherence was enhanced in scaffolds coated with collagen I and fibronectin. Adherent cells stained positively for smooth muscle protein, pancytokeratin and vimentin, consistent with amniocytes2, and no differences in staining were seen with the various bioactive protein coatings. We conclude that gelatin microspheres are as effective a scaffold as gelatin sponges for support of cellular ingrowth and amniotic fluid cell adhesion and that collagen I and fibronectin coatings enhance amniotic fluid cell adhesion to the gelatin based scaffolds.

Discussion and Conclusions

This study supports the potential for application of tissue engineering to early gestational coverage of MMC by ultrasound guided injection. However, the approach requires further optimization because of the unique challenges of the intrauterine environment. Specifically, methods to promote attachment and prevent dispersal of the microsphere scaffold after injection are needed.

References