The Use of Human Amniotic Membrane for Cartilage Repair: A Sheep Study

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Introduction

The repair of cartilage defects in humans can be a difficult endeavor, and multiple options exist for the surgeon to approach this topic. The surgeon may choose to influence the defect with microfracture or abrasion techniques to stimulate a fibrocartilage patch in which to fill the defect. There are also options available that allow for the filling of the defect with chondrocytes of variable sources, both of autograft and allograft origin. The goal with all of these procedures is to replace cartilage defects with cartilage or cartilage-like material that is as close to normal hyaline cartilage as possible. An ideal choice for cartilage restoration and repair would be something that is readily available, easy to place in a defect, and minimal morbidity to the patient.

Multiple studies exist expounding on the potential uses of human amniotic cells in various platforms for tissue repair. It has been proven that amniotic cells are pleripotent in nature and can be influenced to produce various cell lines including chondrocytes.[1] Further, it has been shown in the lab that demineralized bone can influence pluripotent cells to produce chondrocyte and osteoblast type cells.[1] The potential for this knowledge to be used to repair cartilage defects has not been explored to the knowledge of the authors up to this point.

This study evaluates the use of human amniotic membrane mixed with demineralized bone to fill cartilage defects in a sheep model. It is hypothesized that this membrane would be able to fill these defects with chondrocyte-like cells, and that the defects would be filled with hyaline cartilage.

Method

Six adult sheep (less than three years old) where chosen for the study. Each of the sheep was evaluated by a licensed veterinarian and was determined to be healthy and without any limb deformity. Each sheep was anesthetized by a licensed veterinarian and one hind-quarter knee of each was sterilized and surgically exposed. Two cartilage defects were created using curettes, one on the weight-bearing surface of the femoral condyle and one in the trochlear grove (Figure 1). The defects did not violate the subchondral bone. Three sheep were used as control sheep and the defects were left unfilled. Three sheep were chosen to receive human amniotic membrane.

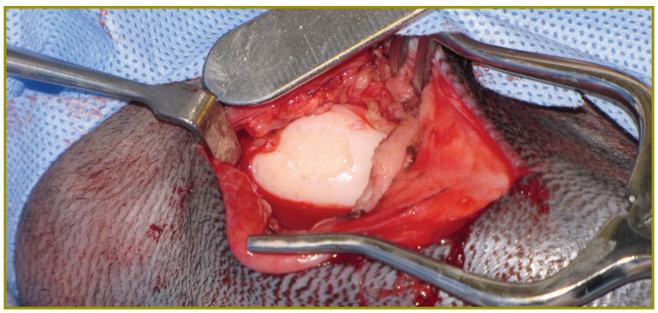

Figure 1: Cartilage Defect

The membrane was procured from a placenta and cut to fit the defect. The portion of the placenta utilized was from the outer membrane of the placenta that has been to shown to have pluripotent cells.[2] Care was taken to place the shiny epithelial layer away from the bone defect because this layer does not have pluripotent cells. The membrane was folded so that the pluripotent cellular layer faced the defect and the joint. Between the layers a small amount of sterilized demineralized human bone was placed in a sandwich-like manner (Figure 2). The membranes were fixed to the defects on the femoral chondyles using micro bone anchors and fibrin glue. The membrane was fixed to the trochlear defects using fibrin glue alone. The wounds were closed and the sheep were allowed to weight bear as tolerated immediately after surgery.

The wounds were evaluated at regular intervals to evaluate for infection and swelling. The sheep were clinically evaluated and a lameness rating was taken at set intervals. At six months post-procedure, the sheep were sacrificed and the distal femurs were harvested. Histological samples were taken of each of the operative sites, both those treated with amniotic membrane and without membrane. Normal cartilage samples were taken from each of the sheep for comparison. The histological samples were taken using mosaicplasty coring instruments to minimize cartilage damage.

The samples from each sheep were evaluated grossly and on a cellular level. Of the sheep that had amniotic membranes placed in their defects, 50% retained the membrane grossly. All of the retained samples were in

the trochlear groove defects. None of the samples on the femoral condyles retained the amniotic membrane. For this reason, analysis of the samples was only performed on those samples that retained the amniotic membrane.

The samples were stained with both H&E and Trichrome staining. Each of the samples (normal, control, and treated) was evaluated using a straight-forward scoring system that is a validated cartilage evaluation method. This evaluation method rates the sample on two levels. First the sample is rated 0-3 on overall appearance (0 = no cartilage present, 3 = mostly normal appearing cartilage) which was referred

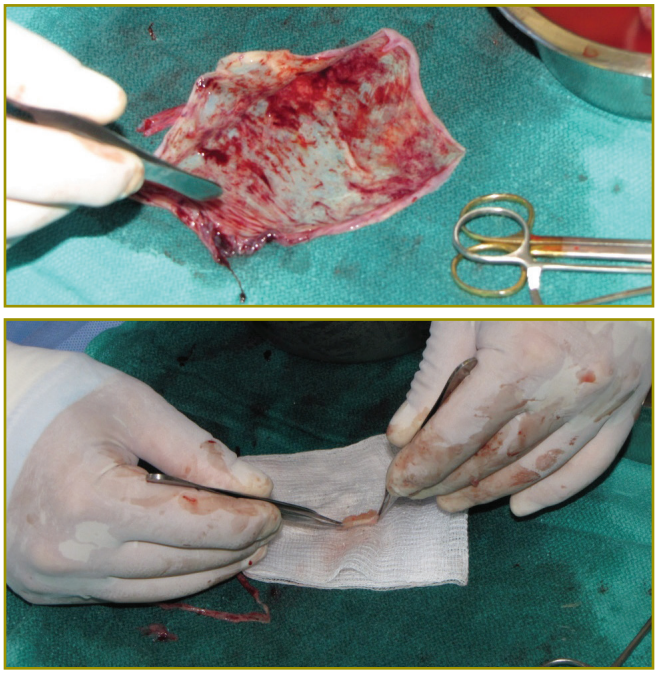

Figure 2: Placental Preparation

to as "simple score". Next, each sample was given a % value of cartilage-like tissue, these values were referred to as "% score". All samples were evaluated by both of the authors who were blinded to the results of the other observer.

Results

The lameness of each of the sheep was evaluated by the veterinarian at set intervals. All but one of the sheep (control) regained full range of motion (ROM) in the limb. All but the sheep that lost some ROM in the limb was rated at a lameness level of 0-1 at final evaluation. Table 1 details the lameness evaluation of each of the sheep.

Of the control sheep, none of the defects filled in with hyaline cartilage or fibrocartilage. In the sheep in which membranes were placed, 50% of the defects appeared to retain the membrane, which is consistent with other similar animal models. Samples of the membrane defects were examined histologically based on a simple, validated scoring system. These samples were compared to normal samples taken from the sheep. There was a strong statistical correlation showing very little difference between the test samples and the normal cartilage. The defects that retained their membranes had evidence of diffuse chondrocyte-like cell proliferation and showed a stromal matrix similar to hyaline cartilage.

Conclusion

Human amniotic membrane is a potential source of plueripotent cells that can be influenced to produce cartilage in defects in sheep model. The implications for application in a human model are promising and warrant further study.

References

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2. Marongiu F, et al. Isolation of Amniotic Mesenchymal Stem Cells. *Current Protocols in Stem Cell Biology*. 2010;1E.5.1-1E.5.11.

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	Evaluation	Lameness (0-5)	Effusion	ROM
Sheep 1 (implant)	2 wks	2	3	
	6 wks	2	0	
	6 mo	0-1	0	full, nml
Sheep 2 (implant)	2 wks	3	2	
	6 wks	2	1	
	6 mo	0-1	0	full, nml
Sheep 3 (implant)	2 wks	2	2	
	6 wks	2	0	
	6 mo	0-1	0	full, nml
Sheep 4 (control)	2 wks	3	3	
	6 wks	1	0	
	6 mo	2	0	
Sheep 5 (control)	2 wks	2	3	reduced flex
	6 wks	2	0	
	6 mo	0-1	0	full, nml
Sheep 6 (control)	2 wks	1	2	
	6 wks	1	0	
	6 mo	0-1	1	full, nml

Table 1