Use of a Surgical Hemostat containing Autologous Plasma, Pepsinized Collagen, and Recombinant Thrombin Modulates Properties of Clot Associated with Subchondral Bone Microdrilling in an Ovine Model

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Disclosures:  
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Introduction: Microfracture, or subchondral bone marrow stimulation, is a common procedure to treat focal cartilage defects and relies on bleeding of the subchondral bone and the subsequent formation of a clot. Cartilage repair may be improved as the initial clot formation may not be complete or uniform since it is left to the natural bleeding of the subchondral bone [1]. We aimed to evaluate whether the use of an autologous plasma-containing hemostat to control bleeding and augment and stabilize the microfracture clot would improve the clot properties in the cartilage defects. The objectives of the present study were to evaluate hemostasis and clot properties in the early stage after microfracture and the use of a surgical hemostat using an ovine model. Microfracture was performed using a novel microdrilling instrumentation aimed to easily and reproducibly create subchondral bleeding.

Methods: Twelve (12) sheep were operated and cared for at Thomas D. Morris (Reisterstown, MD) under IACUC approval #12-038 and GLP quality controls. Two full thickness chondral defects were created in the distal part of the femur: one 8mmx10mm was made in the medial femoral condyle; the other one 6mmx12mm defect was made in the trochlea [2]. Each defect was treated by microfracture/microdrilling (MFX - MicroFX™ OCD, Stryker) and alternatively treated with a surgical hemostat containing autologous plasma, pepsinized microfibrillar collagen and recombinant thrombin (SH - VITAGEL® RT Surgical Hemostat, Stryker). Hemostasis and clot properties were evaluated at Time 0 and after 2 weeks. In four sheep (Time 0), microdrilling defects were created bilaterally. In the remaining eight sheep (Time 2weeks), microdrilling defects were created unilaterally. The hemostat was prepared for each treated defect by first preparing the plasma syringe containing ≈4cc of autologous plasma isolated by centrifugation from 10cc of autologous blood per manufacturer’s instructions for use and assembling the plasma syringe with the collagen/thrombin syringe prior to delivery to the defect. SH was applied to the defect once a bleeding bed was achieved in the defect in sufficient volume (≈0.2-0.4cc) to fill the entire defect, and was then allowed to congeal. The joint was closed once bleeding was completely stopped. The sheep were placed in a Thomas splint for the full length of the 2-week live phase.

Hemostasis was measured (N=16) for all defects as the time it took for bleeding to stop after delivery of the hemostat to the microdrilled defect. Clot/defect fill (N=8) was visually evaluated (non-instrumented). Clot/defect stiffness (N=8) was assessed semi-quantitatively using a flexible #10 scalpel blade probe with a 0-4 scoring relative to the surrounding intact cartilage (0 = no stiffness, <10% compared to cartilage; 1 = very soft, <25% cartilage; 2 = soft, <50% cartilage; 3 = semi-hard, <75% cartilage; 4 = cartilage like, ≥75%)

Results: Subchondral bone bleeding/marrow stimulation was achieved in all animals. Time to hemostasis was significantly reduced by 52% when SH was used with MFX (369 vs. 776 seconds, p<0.01, Fig. 1). SH clots were significantly stiffer once hemostasis was achieved (Time 0, 1.9 vs. 1.0, p<0.001), and continued trending to greater stiffness 2 weeks post-op (Time 2wks, 2.8 vs. 2.4, p=0.12, Fig. 2). Defect fill was significantly increased 2 weeks post-op when SH was applied (64% vs. 49%, p<0.05, Fig. 3). Visually, the majority of SH clots appeared glossy white, suggesting fibrocartilage formation. One SH sample was noticeable as the clot could not be harvested because the repaired tissue was integrated with the surrounding cartilage and subchondral bone. That sample showed histological evidence of fibrocartilage and collagen II histochemistry. Overall clot histochemistry at 2 weeks showed preliminary, incomplete maturation of the clot with no apparent organized structure, as expected after this short in vivo period. As previously reported [3], results in the trochlea defects were slightly better than in the condyle. An increase in GAG content was visually observed in some samples, with a higher frequency in the SH samples. Again, one SH sample was noticeable as the clot histology exhibited a substantial matrix formation surrounding cells with evidence of lacunae formation, suggesting the early stage of fibrocartilage formation (Fig. 4). Biochemistry analysis showed an average increase in GAG content after 2
weeks in both treatment groups, with no differences between the two treatment groups. Collagen I and II biochemistry was inconclusive, likely due to the lack of sensitivity of the kits used for analysis. RT-PCR analysis showed an increase in Collagen I, and to a lesser extent in Collagen II, after 2 weeks in vivo. The signal intensities exhibited a high degree of variability, although these signals appeared to be more consistent in the SH group. When samples were compared in the same animals (N=2), the SH group exhibited higher Collagen I and II signal intensity than the MFX group. Aggrecan analysis was inconclusive.

**Discussion:** Overall, these results showed that microdrilling was an efficacious technique to produce subchondral bleeding/marrow stimulation and form clots in this ovine model. The addition of a surgical hemostat containing autologous plasma, pepsinized microfibrillar collagen, and recombinant thrombin showed preliminary benefits in time to hemostasis, clot stiffness, and defect fill. When the hemostat was used, repair tissue contained more cells, more collagen and suggested an earlier formation of fibrocartilage when compared with control defects where microdrilling alone was used. This study is being prolonged to 24 weeks to evaluate the potential long term benefit of using a hemostat as an adjunct to microdrilling.

**Significance:** Microfracture (subchondral bone marrow stimulation) is a common procedure to treat focal cartilage defects and relies on the properties of the clot formed within the defect. This approach may be improved by using a surgical hemostat to control bleeding within the perimeter of the defect and collaterally modulate clot properties. Under the conditions of the present animal study, the use of a hemostat containing autologous plasma, pepsinized microfibrillar collagen, and recombinant thrombin in microfracture/microdrilling defects provided hemostasis and control of the blood clot but also suggested improved properties of the clot. While microdrilling alone resulted in some positive outcome due to substantial and uniform bleeding for all defects, the amount of clot tissue in the defect, clot stiffness, clot biochemistry and cellular organization were all improved when the hemostat was used.

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Cartilage, 16(S4), 2008.
Figure 1. Time to hemostasis at time of surgery for all treatment groups (N=66).

Figure 2. Clot stiffness (semi-quantitative) at Time 0 and 2 weeks for both MFX and SH groups.

Figure 3. Defect Fill (non-instrumented visual evaluation) observed after 2 weeks.

Figure 4. Histology (Safranin O staining) of one unique SH clot sample.