Introduction
Growth factors are known to be involved extensively in wound healing. Tissue repair starts with a highly regulated sequence of events including chemotaxis of inflammatory cells and fibroblasts, angiogenesis, cellular proliferation and production and remodeling of extracellular matrix. Coordination for this process is achieved by autocrine and paracrine actions of growth factors which serve to communicate signals from cell to cell and between cell and matrix (Kang, et al.). Frequently noted growth factors early in the wound healing process are transforming growth factor beta (TGF-beta), Insulin-like growth factor 1 (IGF-1) and Epidermal growth factor (EGF). Little is known, however, about the timing of the appearance of these factors in the normal healing tendon (Ngo et al). In this study we used immunofluorescence staining to observe the appearance of these three factors in the first week after laceration and repair in an in vivo canine model.

Methods
Our study evaluated tendon healing in eight adult mongrel dogs. A laceration was made in the fourth digit of each forepaw flexor tendon with the digit in full extension. The laceration was located at a clinically relevant point where the pulley and the tendon repair interact during flexion. The lacerations were repaired with a modified Kessler suture of 4-0 looped cable type supramid (S Jackson, Arlington, VA) and a running suture of 6-0 nylon (Ethicon) and the operated limb was then immobilized with a combination of casting and radial neurectomy (to prevent weight bearing). The tendons were harvested at 1, 3, 5 or 7 days after the surgery. Each group consisted of tendons from two separate dogs. Controls from the respective mobile digits on the contra lateral paws were also taken. To minimize the effects of surgery-related inflammation, tendon within 7 mm proximal and distal to the operated site was discarded. The tendons were paraffin embedded and cut at 4 micrometers. Tissue sections were deparaffinized and rehydrated in Xylene and passed through a graded series of ethanol and distilled water prior to staining. Antigen retrieval with Citrate Buffer Solution (Zymed Laboratories, San Francisco,CA) for 20 minutes and cooling down of 5 minutes gave the best results. Specimens for immunofluorescence study were incubated with mouse monoclonal antibodies for 1 hour at room temperature with EGF (Lab Vision Corporation, Fremont, California, USA ), IGF-1 (Diagnostic Systems Laboratories Inc., Webster, Texas, USA ) and TGF-beta (Promega, Madison, WI) at a dilution of 1:100 in Antibody Dilution Buffer (ChemMate, Ventana Medical Systems, Tucson, AZ). For each section there was a negative control of mouse monoclonal IgG at the same dilution as the primary antibody (Clone NCG01, NeoMarkers, Labvison, Fremont,CA). The anti-mouse fluorescent secondary antibody (AlexaFluor, Molecular Probes, Eugene, OR), at a dilution of 1:800, had a wavelength of 488 nm. Hoechst counterstaining was used to observe the nuclei and with that the cellular response of the tendon. Confocal microscopy (LSM 510, Carl Zeiss Inc) was used to note the location of TGF-beta, EGF and IGF-1 staining for each tendon. The confocal microscope images were saved in an image capture database.

Results
Each of the four time points demonstrated expression of IGF-1, with no expression of either EGF or TGF-beta at any time point. The Hoechst counterstaining demonstrated cellular proliferation in the epitenon, especially after day 5. Staining intensity of IGF-1 was already seen on day 1 (Fig. 1a) until day 7. The IGF-1 was first tightly seen in endotenon and diffuse in the epitenon. On day 7 the expression had a particular staining distribution and was located in the epitenon where it formed a line underneath the thicker layer of nuclei (Fig. 1b).

Discussion
Little is known about the expression of growth factors normally in a healing tendon. Ngo et al studied the expression of TGF-beta in healing tendon in a rabbit model and found that expression peaked at 14 days and tapered off by 56 days. Little expression was noted in the first week, consistent with our findings. Kang et al. have shown that IGF has a strong stimulating effect on rabbit tendon cells in culture. Invivo studies can be used though as a working mechanism but not for timing in an invivo study. Jann et al have shown that exogenous EGF stimulates migration of chicken tendon cells in culture. Here, like Ngo et al, we chose to look at early growth factor expression in vivo. The methods of staining were repeatable and the results were consistent among the two tendons of each day. It seems clear that IGF expression begins very early in the course of canine tendon healing. Additional studies are required to determine the time of expression for EGF and TGF-beta; clearly these may be expressed after 7 days, as noted for TGF-beta by Ngo et al. It will also be important to correlate growth factor expression with specific cellular events associated with replication, apoptosis, and matrix synthesis. Our data provide evidence that IGF-1, a potent growth factor know to increase the healing potential of tenocytes in culture and stimulator for collagen synthesis (Abrahamsson et al.), is the first to be expressed in the healing process of canine flexor tendons. The characteristic localization suggests that IGF expression, which is noted as early as day 1, might be one of the signals stimulating epitenon cell proliferation, which is noted on day 5.

References

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