THE EFFECT OF MUSCLE AND FASCIOCUTANEOUS TISSUE ON ANGIOGENESIS IN OPEN TIBIAL FRACTURE HEALING

Introduction: Open fractures of the leg are a reconstructive challenge. Early restoration of the soft tissue envelope has dramatically improved the outcome of these fractures with regard to deep infection and function. However, the ideal tissue for covering open fractures remains controversial. Muscle is said to be superior but the mechanism is pivotal and other factors such as fibroblast growth factor 2 (FGF-2), Vascular endothelial growth factor (VEGF) has been shown to be unclear. A higher blood flow was thought to be the key factor although this has not been established. Angiogenesis is vital for fracture healing. Vascular endothelial growth factor (VEGF) has been shown to be pivotal and other factors such as fibroblast growth factor 2 (FGF-2), insulin-like growth factor (IGF-1), and platelet-derived growth factor (PDGF-BB) have been implicated.

We have developed a novel murine open tibial fracture model to compare the vascularity of muscle and fasciocutaneous tissue and investigate their role in angiogenesis during fracture healing.

Method: An open tibial fracture, stripped of periosteum, was created in female C57Bl/10 mice, under Home Office Approval. Skeletal stabilisation was achieved with a 0.38mm diameter intramedullary pin. Animals were divided into experimental groups which allowed exclusive comparison of the soft tissues. A piece of sterile, inert material (Polytetrafluoroethylene, PTFE), was inserted at the fracture site to exclude either muscle posteriorly (Fasciocutaneous group) or skin and fascia anteriorly (Muscle group). A control group was devised consisting of fracture plus stripping but no PTFE; the Skeletal Injury only group (Fig 1).

Animals were harvested at days 3, 5, 7, 9, 14, 21 and 28 days post-fracture and healing was assessed histologically. Bridging of the fracture site, by callus containing woven bone was quantified by histomorphometry. Immunohistochemistry was performed on specimens, to estimate vascularity using an antibody to factor VIII related antigen, which selectively demonstrates vascular endothelium. Vascular densities were determined within the muscle and fasciocutaneous tissues adjacent to the fracture site, by manual counting of immunostained vessels at high magnification (x500) using an eyepiece graticule. Angiogenic factors were measured by ELISA in tissue specimens and the level of soluble VEGF Receptor 1 (sFlt-1), a negative influence on new blood vessel formation, was also assayed. All statistical analyses were performed by 2 Way ANOVA.

Results: Fracture healing was more advanced beneath muscle, with earlier bridging of the fracture site by callus (Fig 2).

Histomorphometry demonstrated higher total bone area at the fracture site in the Muscle group (and Skeletal Injury control; 1.2±0.4 x10² and 1.2±0.3 x10² µm² respectively) compared to the Fasciocutaneous group (0.7±0.2 x10² µm²). However, significantly greater vascular densities per unit area were observed in fasciocutaneous tissue compared to muscle (p<0.0001) at all time points during fracture healing (Fig 3).

Quantification of VEGF, sFlt-1, FGF-2, IGF-1 and PDGF-BB showed the variation of these factors during the phases of fracture healing. Levels within fasciocutaneous tissue were higher throughout, although this difference was only significant with VEGF (p<0.05) and IGF-1 (p<0.0001). Levels of sFlt-1, FGF-2, IGF-1 and PDGF-BB were significantly different over time (data not shown). The relative excess of VEGF over sFlt-1 receptor was determined, to investigate the angiogenic drive within the tissues, and was found to vary significantly with time, peaking at day 14 post fracture (Fig 4).

Discussion: We have studied the exclusive effects of muscle and fasciocutaneous tissue on angiogenesis during the healing of open tibial fractures stripped of periosteum. Faster bridging was demonstrated beneath muscle compared to skin and fascia alone, comparable to the control. However, there were significantly higher vascular densities in fasciocutaneous tissue compared to muscle. The temporal distribution of factors known to be pivotal for the angiogenic process has been determined within the soft tissues, and correlates with the pattern of vascularity. This confirms the importance of the soft tissues in establishing the biological milieu of the fracture microenvironment necessary for healing. Our results contradict the widely held view that muscle provides superior coverage of open fractures because of a higher capillary density. The data suggest that vascularity is not the only factor influencing the choice of soft tissues. Extrapolating our data to clinical practice, muscle should be the tissue of choice for covering high-energy open distal tibial shaft fractures.

References

Affiliated Institution for Co-Authors
**Charing Cross Hospital, London, UK
***Bioengineering Department, Imperial College, London, UK
#Lund University, Sweden
^ Hand Surgery and Peripheral Nerve Injuries Unit, Royal North Shore Hospital, Sydney, Australia

52nd Annual Meeting of the Orthopaedic Research Society
Paper No: 0067