Transplanted Abdominal Granulation Tissue Induced Bone Formation – An in vivo Study in Sheep

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Introduction: One of the biggest limitations of tissue engineering is the inability to provide instructive microenvironments during cell transplantation. This leads to uncontrolled differentiation of cells in the wound, a lack of vascular in-growth and fibrous tissue formation. The target microenvironment for most tissue engineering applications is a wound healing environment. Wound healing is traditionally broken down into three main stages, the inflammatory phase, the proliferative phase and the remodelling phase. Following the initial injury, disruption of vasculature and formation of the blood clot, the wound site experiences an infiltration of leukocytes and macrophages aimed at removal of necrotic tissue and foreign bodies, as well as releasing angiogenic, migratory and proliferative cytokines. During the early stages of the subsequent proliferative stage, formation of granulation tissue occurs simultaneously with neo-angiogenesis. The formation of a stable, vascularised granulation bed supports the influx of progenitor cells. These cells are differentiated by a series of environmental cues and begin to lay down extracellular matrix typical of their phenotype. Blood vessels and tissue begin to mature and the remodelling of the wound site begins. It is commonly acknowledged that bone heals by these mechanisms.

Recently it has been shown that the implantation or transplantation of the cells and microenvironment found in granulation tissue can be used to aid in the formation of differentiated tissues[1,2] including skin and blood vessels and Hoenig et al [2] have shown that a low morbidity source of granulation tissue can be harvested from the peritoneum.

This study aimed to examine the effect of transplanting autologous peritoneal granulation tissue to a bone defect.

Materials and Methods: In the treatment group 3 mixed bred ewes were operated on in a 2 stage procedure. In stage one a polyanhydride rod 15mm length and 4mm diameter, drilled with 2mm holes was placed in a polyvinyl chloride capsule with 2mm holes drilled through it (Figure 1(a)). Three capsules were implanted into the peritoneal cavity and attached to the peritoneal wall through a vertical incision in the left para-lumbar fossa in each animal.

Stage two took place 2 weeks later, when the capsules were retrieved. One capsule was kept for histology, in the second the PVC outer casing was removed and Hoenig et al [2] have shown that by transplanting the microenvironment observed during early stage abdominal adhesion formation that it was possible to mimic the granulation stage of fracture repair. By recapitulation of this stage it was possible to induce the formation of bone in a defect.

Secondly this study highlights the importance of providing an appropriate microenvironment. Successful tissue regeneration relies on local environmental cues acting on viable cells in appropriate microenvironments. This study showed that by transplanting the microenvironment observed during early stage abdominal adhesion formation that it was possible to mimic the granulation stage of fracture repair. By recapitulation of this stage it was possible to induce the formation of bone in a defect.

The importance of microenvironments to tissue cannot be overstated. This study was the first to show that abdominal granulation tissue can be used to form bone.

Discussion: This study highlights two important factors about early wound healing microenvironments that are relevant to tissue engineering strategies.

The first is that the early wound healing response is generic. Previously there have been very few reports in the literature of the transplantation of autologous microenvironments. Winter and Hass reported a technique of granulation tissue transplantation from the wound edges to a central non-granulated region as a method of decreasing the time to wound closure and improving the cosmetic result[1]. Arterial tissue has been repaired using granulation tissue grafts[2] and granulation tissue encapsulated stents[3]. In this study we have demonstrated that granulation tissue can be used to form bone.

Results: Histology showed that the tissue obtained from the peritoneum, was a granulation type tissue consisting predominantly of macrophages and fibroblast-like cells laying down a loose connective matrix. The Van Gieson’s stain also indicated the presence of collagen (Figure 1(b)). In the two control animals no bone formation was observed, there was no sign of chronic inflammation and no evidence of polymer degradation (Figure 1(c)). In all three treatment animals, CT and histology show formation of bone adjacent to the implant site, indicating involvement of the peritoneal granulation tissue in the osteogenic reaction (Figure 1(d)).