Report for the Australian and New Zealand Head and Neck Cancer Society Foundation Grant 2022 (Reconstructive Surgery Fund)

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Title: **Bio-printed adipose-derived stem cells for engineering vascularized bone tissue within an in vivo bioreactor**

Project Details

Description:

The study advocates for an innovative approach to address the challenges of mandibular reconstruction by utilizing bone tissue engineering (BTE). The strategy involved employing adipose-derived stem cells (ADSCs) in combination with a 3D printed polyether ether ketone (PEEK) bioreactor chamber and Gelatin Methacryloyl (GelMA) hydrogel. ADSCs, known for their self-renewal capacity, were chosen for their ease of isolation and applicability for autologous use. The 3D culture system,

incorporating a PEEK bioreactor chamber and GelMA hydrogel, offered a mechanically stable platform for cell encapsulation and survival. Additionally, PEEK bioreactor was treated with Plasma Immersion Ion Implantation (PIII) to enhance its cellular adhesion property allowing better cell attachment and growth. This comprehensive approach demonstrated the potential role of osteogenically differentiated ADSCs, encapsulated into GelMA in generating ectopic vascularised bone within the PEEK bioreactor chamber, with simultaneous stimulation, either from underlying scapular bone or from overlying scapular periosteum.

Aims of the project:

1. The primary aim of this study is to generate vascularized bone tissue from GelMA-ADSC constructs placed within a 3D-printed PIII-treated PEEK bioreactor.

2. The secondary aim is to compare three sites of ovine implantation: scapular periosteum, scapular bone, and subcutaneous adipose tissue.

Results

The experiment involved harvesting bioreactor samples from sheep scapula 10 weeks post-implantation. Following the harvest, tissue biopsies were promptly collected from each bioreactor sample and preserved through snap-freezing for subsequent molecular assays. Subsequently, each bioreactor underwent microCT scanning to assess the volume of newly formed bone tissue (BV). The samples were then fixed in 10% neutral buffered formalin (NBF) for further processing, including histological and immunohistochemical tissue sectioning. This involved staining with hematoxylin and eosin (H&E) stains, application of primary antibodies targeting osteogenic and angiogenic markers, and subsequent microscopic analysis. Clinical pathologists evaluated H&E stained tissue sections to assess new bone tissue formation in different groups (GelMA only vs GelMA-ADSCs, scapular bone vs periosteum contacting bioreactors).

MicroCT analysis revealed mineralized tissue growth in both GelMA only control and GelMA-ADSCs groups, in both upper (periosteum contacting) and lower chambers (scapular bone contacting). While no significant difference in BV was observed between GelMA only control and GelMA-ADSCs groups regardless of the chambers

(upper or lower), lower chambers (scapular bone contacting) exhibited significantly higher BV than upper chambers (periosteum contacting) in both GelMA only control group (difference between means ± SEM0: 0.008242 ± 0.002430), and GelMA-ADSCs group (difference between means ± SEM0: 0.01042 ± 0.001825). Histological evidence demonstrated vascularized neo-bone tissue growth in both GelMA only and GelMA-ADSCs groups. Notably, immunohistochemistry (IHC) and molecular assays are pending and anticipated to be conducted soon, with the outcomes to be reported subsequently.

Conclusions

In conclusion, the study has thus far shown that GelMA-ADSCs constructs within the PIII-treated PEEK bioreactor chamber successfully generated vascularized new bone tissue, as indicated by microCT and histological results. Further analysis of the newly grown bony tissue will be carried out using IHC and quantitative real-time polymerase chain reaction (qRT-PCR)-based molecular assays.