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Chapter 11

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Oral applications of different grafting technologies and materials include periodontal regeneration (Richardson et al. 1999; Velasquez-Plata et al. 2002; AlGhamadi et al. 2010) , peri-implantitis (Schwarz et al. 2006), sinus elevation to enable implant placement (Jensen et al. 1996; Yildirim et al. 2001), and bone regeneration following tooth loss due to trauma or alveolar ridge preservation. Grafting materials may be placed into a tooth socket prior to implant placement (Vittorini Orgeas et al. 2013; Avila-Ortiz et al. 2014; Atieh et al. 2015) or accompany immediate implant placement if there is a disparity between implant and tooth socket diameters (Araújo et al. 2011), or be used to cover exposed implant surfaces when there are deficiencies of alveolar bone (Hämmerle et al. 2004). Even when a autogenous block bone graft is used to augment a healed, deficient alveolar ridge, this is often accompanied by the use of bone replacement grafting materials and/or resorbable membranes (Cosyn et al. 2013).

The development of guided tissue regeneration using membrane technologies to regenerate firstly periodontal tissues (Pontoriero et al. 1988) and then bone (Dahlin et al. 1988) led to the concept of guided bone regeneration, either prior to or contemporaneous with implant placement (Nyman et al. 1990). This evolved over forty years from non-resorbable to resorbable membranes (Hurzeler et al. 1996) with bone replacement grafting materials (Zitzmann et al. 1997) and bioactive substances (Sharma and Pradeep, 2011; Lekovic et al. 2003; Miron et al. 2016); this history has recently been comprehensively reviewed (Scantlebury and Ambruster, 2012). Research continues with a view towards the development of so- called “third generation” biofunctionalized membrane and grafting technologies (Sam and Pillai, 2014), with an eye towards fourth-generation technologies where appropriate carriers may be engineered for the transplantation of mature periodontal ligament cells (Ishikawa et al. 2009) or appropriate multipotent “stem” cells (Bartold et al. 2006). In a review of this therapeuticapproach, Monsarrat et al. (2014) commented that “the challenge remains to identify the best combination of cells, biomaterials, and biomolecules for various clinical situations, using animal models that best represent the

etiopathophysiology" of the human clinical situation.

Clinical translational research requires a multidisciplinary team approach and involves multiple steps from initial product concept to human phase 1 clinical trials (Figure 1). A key part of this is the use of animal models to validate in vitro findings; such preclinical work provides the supporting base for the so-called "evidence pyramid' (Varoni et al. 2015). In New Zealand we have a long history using sheep as an animal model, for examination of regenerative periodontal therapy, initial implant healing, stem cell therapy, sinus grafting, peri-implantitis and alveolar ridge preservation; this work has been presented at previous meetings of the Asian and Pacific Society of Periodontology (Duncan, 2014, 2016; Duncan et al. 2018). Our lab uses four main sites in sheep, each reflecting different conditions in humans: the healed edentulous mandibular ridge, the maxillary sinus, the femoral epicondyle, and mandibular tooth sockets (Figure 2). We have used these sites for testing commercially-available products as well as for our own experimental work developing new materials. Our experimental designs attempt to address various considerations that dictate the possibility of translating our work, from laboratory to chair-side use by dental practitioners (Figure 3).

Building on the work of Lindhe and colleagues in the dog tooth socket model (Cardaropoli et al. 2003; Araújo and Lindhe, 2005) we established a similar model using sheep mandibular premolar sites. We then used this large animal model for testing a novel regenerative biomaterial that consisted of tricalcium phosphate (TCP) nanoparticles, flame-spray synthesised from calcium hydroxide and electrospun with poly(lactide-co-glycolide) (PLGA) at a ratio of 2:5 (40% TCP) into 10 micron fibres. This formed a flexible, highly- porous cottonwool-like material. Previous testing by the laboratory that developed the material, showed encouraging results in a small animal (rabbit) cranial defect model (Schneider et al. 2008). In our lab, comparison with sheep tooth sockets grafted using a bovine bone xenograft and cross-linked collagen membrane, demonstrated comparable results after 18 weeks healing, but without the presence of residual un- resorbed grafting material in the tooth sockets (Liu et al. 2016). Furthermore, these results confirmed the utility of this animal model. Simultaneous grafting into a peri-implant critical-size defect in the femoral epicondyle of the same animals also provided valuable information. However, one drawback of this material was the extremely slow process required for production, making it unlikely that this could be scaled-up for commercial production.

Subsequent experiments focused on testing of another novel material in 11 sheep, again with simultaneous use of two sites (mandibular premolar sockets and maxillary sinus).

The tested material consisted of a commercially-availablecone-shapeddevice made of type I equine collagen, reinforced with biphasic calcium phosphate granules (60% hydroxyapaptite (HA) and 40% TCP) ((Parasorb ConeOss®, Resorba Medical GmbH). Controls included un-grafted tooth sockets and bovine xenograft with collagen membrane (BioOss® and BioGide®, Geistlich Pharma New Zealand Ltd.). After 16 weeks, histomorphometric analysis showed equivalent results comparing the test materials against BioOss® for formation of new bone, both in tooth sockets (Lander, 2016) and in the maxillary sinus

(Sheftel et al. 2019), however unlike BioOss®, the test material did not prevent alveolar ridge atrophy. In this experiment, dental implants were not placed into the grafted sinus sites, however the results we achieved with both the test material and the bovine xenograft were comparable to our previous work (Phillip et al. 2014), where implants were placed into sheep maxillary sinus with simultaneous grafting using a synthetic alloplastic material consisting biphasic calcium phosphate particles, 60% HA and 40% beta-TCP (Straumann® Bone Ceramic, Institut StraumannAG, Basel, Switzerland).

Our current work has evolved from two streams of investigation. Building on previous work with dental restorative materials that incorporated nano-silver (Garden et al. 2013), we developed a nano-silver antibacterial gel that we tested for safety and efficacy in a split-mouth periodontitis/peri-implantitis model in sheep (Duncan et al., 2018.) Simultaneously we had been working on a novel bovine xenograft material called MoaBone® (Molteno® Ophthalmic Ltd., Dunedin, New Zealand), a by-product of the milling of hydroxyapatite spheres used for implantation into eye-sockets following orbital enucleation (Jordan et al. 2000; Suter et al. 2002). Implantation of these highly-processed bovine hydroxyapatite granules into our sheep sinus model resulted in comparable histomorphometric outcomes to the BioOss® controls, however the test material showed greater osteoclastic resorption; it was apparent that this material needed further optimisation in order to make its handling characteristics and resorption times acceptable for clinical use (Smith et al. 2018).

We have now combined our patented nano-silver technology (Cotton et al. 2015, 2017) with the optimised MoaBone® in both particulate and block forms, with a view towards developing a xenograft that is resistant towards infection, without the need for antibiotics. Our initial in vitro work has shown that silver nano-particles (AgNP) are bacteriostatic in low concentrations against S. mitis, S mutans and E coli, and less cytotoxic towards cultured human gingival fibroblasts than chlorhexidine or silver diamine fluoride. The bacteriostatic nature and low cytotoxicity of AgNP shows promise for its potential use as an antimicrobial agent in bone grafting applications. We have published results showing the safety and efficacy of our silver nanoparticle technology (Gee et al. 2018) and are now working on the optimisation and biofunctionalization of MoeBone® xenograft blocks, the in vitro assessment of AgNP toxicity towards osteoblasts and osteoclasts, and the development of a hydrogel incorporating AgNP loaded hydroxyapatite particles, with the intention of testing these in vivo in a small animal model (rabbit cranial CSD) in the near future.

Whilst we feel that our AgNP-xenograft has potential for clinical use, we recognise that the long term goal will be the development of cellular-based solutions for bone and tissue regeneration. In our lab, we have worked with adipose derived stem cells (Godoy Zanicotti et al. 2017; Zanicotti et al. 2018) and periosteal-derived stem cells (Naung et al. 2019), but at present we consider dental-pulp derived cells to have the greatest potential (Coates et al. 2019). Currently we are working to extend our findings using 3-D printable hydrogels, with a view towards bioprinting appropriate scaffolds containing dental pulp cells for oral regeneration.

Conclusions

Guided tissue and guided bone regeneration are successful clinical treatment options, widely used to regenerate bone and periodontal ligament around diseased teeth and implants, to preserve bone in tooth sockets and to regenerate lost bone prior to implant placement. However, currently-available therapies for GTR and GBR largely involve passive scaffolds and/or tissue excluding membranes. The next generation of membranes and grafts will involve bioactive materials, which need further development. A focus on cell-promotion and anti-infection should be priorities for these next-generation products. The "next-after-next" therapeutic treatments are likely to involve personalised 3-D printed constructs carrying the patient's own cells. Each step in the development of the technologies requires multi-skilled teams working with appropriate preclinical in vitro and in vivo models in order to demonstrate safety, efficacy, scalability and cost-benefit, prior to commencing human clinical trials.

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