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Abstract. The impact of Tony Melcher's scientific work on periodontal regeneration is discussed in light of his interests in cellular domains and bone healing, the physiology of the periodontal ligament, and his development of organ and cell culture methods. Collectively, his scientific accomplishments have pointed us in new directions for approaching periodontal regeneration and have helped to create new research opportunities, many of which were previously unrecognized. Tony's scientific ideas are insightful, numerous, and compelling; his ability to make these ideas come alive and relevant to clinical problems has been a major influence in shaping our current thinking on the biology of the periodontium and on periodontal regeneration.

Key words: periodontium, bone, cell culture, wound healing, periodontal ligament.

Introduction

Attempts to regenerate periodontium lost as a result of disease continue to hold the attention of dentists, dental scientists, and the public. The problems of achieving periodontal regeneration are legion, and despite heroic efforts, progress up until only recently has been frustratingly slow. Indeed there is a rich history of the art, science, and quackery of periodontal interventions, some of which stretch into the dim past; new approaches not yet envisioned will undoubtedly extend well into the next millennium.

In the latter half of the 20th century, Tony Melcher (Fig. 1) has made fundamental observations into the nature of periodontal tissues and the regulation of the cells that synthesize and remodel these tissues, a pursuit that, with some digressions, has occupied him throughout much of his scientific career. His experimental approaches and use of some fascinating model systems have considerably advanced our understanding of the basic problems inherent in periodontal regeneration. Tony's experiments have suggested new ways of thinking and approaching an incredibly difficult challenge: How do we regenerate a normally functioning periodontium? Notably, Tony's insistence on rigorous application of the scientific method has helped propel studies of the periodontium into the forefront of dental sciences. This brief retrospective considers ground-breaking aspects of his scientific work that have had an impact on guided tissue regeneration, the use of cell culture for study of periodontal cell physiology, and the central role of the periodontal ligament in periodontal regeneration.

Some history

No less a luminary than the 18th century scientist and surgeon John Hunter was fascinated by the challenges and possibilities of transplanting teeth and restoring the lost fibrous attachments and bone that are destroyed by periodontitis. Some of Hunter's experiments (which can be viewed in the Hunterian Museum in The Royal College of Surgeons of England in London, England) focused on

transplantation of teeth into such unusual sites as the coxcomb of a rooster! Hunter thought that wound healing and developmental processes shared many similarities, and an examination of his experimental work today clearly shows his strong interest in the biology of dental tissues. However, the realization that a biological approach to studies of periodontal regeneration would be most fruitful has not always been appreciated by subsequent scientists working in this field. Indeed, until relatively recently, the aim of restoring lost periodontal tissues was restricted to restoring lost alveolar process. A reading of the periodontal regeneration literature of the mid-1950s shows that there was increasing interest in this approach. For example, the work of George Cross in London, England, on the use of graft materials to improve the results of periodontal surgery is notable for its biological approach. What is striking about much of the clinical literature is how little biology was being considered. In effect, what was being attempted was the filling of holes in the alveolar bone with various types of materials, an idea that is analogous to filling a cavity in a tooth. Attempting to do just that is where Tony Melcher made his start.

Early days

Tony was born into a family with a strong dental background. His father was a dentist, and Tony attended dental school at the University of the Witwatersrand in Johannesburg, South Africa. After graduation, he practiced in his father's clinic and developed a strong interest in periodontics, although he realized that there was more craft than knowledge in periodontology at that time. His clinical interests began to expand beyond the dental office, and he began research training under the tutelage of the late James Irving at the University of the Witwatersrand. Irving was an expert in the physiology of mineralization and, interestingly, later left South Africa to continue his work at the Forsyth Dental Center in Boston. He was instrumental in providing Tony with laboratory space and in encouraging him to consider research questions. Thinking that anorganic bone could be useful for periodontal bone grafting, Tony aimed at comparing the efficacy of anorganic bone with that of other types of graft materials. Together, they used a rat femoral model to ask which of anorganic bone and other types of grafts could best be expected to regenerate bone in a circumscribed wound (Melcher and Irving, 1963). Perhaps more importantly, they asked, How do these grafts work? As part of this study, Tony examined, with Irving and also with Jan Dreyer, the nature of bone healing in experimental defects in rat bone. In one of the experiments, Tony found that protecting the clot in the wound with a domed shield permitted the callus to form a protuberance on the bone surface, which did not happen in the absence of the shield (Melcher and Dreyer, 1962). This experiment was based on the work done earlier by J.J. Pritchard in Belfast, Northern Ireland, who believed that osteogenic cells function in spatially discrete domains. Pritchard thought that preservation of the osteoblastic domain in a bone wound and the exclusion of soft connective tissue elements from

Figure 1. Tony Melcher at work in his office at the University of Toronto.

this domain are critical to the filling of the defect with new bone. Tony proposed that the role of the shield was to exclude the overlying soft tissues from the domain protected by the shield, so allowing osteoblasts to migrate into the area and to deposit bone there. So, it is evident that the filling of bone defects was not just like filling a hole in a tooth; instead, it was a biological puzzle that involved interaction between osteoblasts and fibroblasts and that, if better understood, could be manipulated to improve clinical outcomes. This concept must have hovered in his mind, because it re-appeared in relation to the regeneration of the periodontium more than a decade later. Some of Tony's early work has a modernistic tone: For example, one of the papers describes the use of Teflon as an implant material (Melcher and Dreyer, 1961).

New horizons

Tony left South Africa for Britain in 1961 to pursue graduate work and to expand his opportunities for research. It appears that it was in London that the seeds for his investigation of the periodontium were sown. While he was working at the Royal College of Surgeons of England in London, three events seem to have had a particularly important influence on him: First, the pioneering work in organ culture being conducted by Dame Honor Fell, then the director of the Strangeway's Laboratory in Cambridge, drew his attention to the significant and inherent limitations

of sole reliance on animal models for obtaining compelling insights into the physiology of cell regulation. Also, there were advantages of complementing *in vivo* studies with those done *in vitro*. As a result, he started to collaborate with Giselle Hodges, who worked at the Imperial Cancer Research Fund. He learned from her the techniques of organ culture. Their work together over a number of years on *in vitro* culture of embryonic mouse mandible demonstrated to him the practicality of culturing mixed epithelial and connective tissues in organ culture in a defined chemical medium (Melcher and Hodges, 1968). This experience ultimately led to an attempt to maintain whole adult periodontium in an organ culture system (Melcher *et al.*, 1973; Yen *et al.*, 1979). It was in England, too, that he was introduced for the first time to the *in vitro* culture of cells and to the promise of being able to manipulate a single experimental variable in a well-defined *in vitro* environment. He had by now also come to understand that the traditional histological *in vivo* approach to studying the periodontium in particular and wound healing in general could not lead to the insights necessary to obtain a mechanistic understanding of basic biological and healing processes. Second, his interactions with John Eastoe, a colleague at the college and a widely renowned analytical biochemist, impressed upon him the value for dentally trained scientists working with collaborators who were non-dentally-trained PhDs. The advantage of this for the training of dentally trained graduate students was to become evident later on when he moved to Canada. Finally, he saw a need to gather the scientific knowledge of the periodontium that was then available. This led him to collaborate with Bill Bowen in editing a contributed volume, The Biology of the Periodontium (Melcher and Bowen, 1969), a widely quoted reference work that served as the standard in this field for many years.

A move to the Great North

Perhaps it was these events as much as anything else that prompted him in 1973 to establish, at the University of Toronto, the Medical Research Council Group in Periodontal Physiology. Tony had moved to Canada in the late 1960s and, along with a number of other investigators from the United Kingdom, including Richard Ten Cate, Dennis Smith, and George Beagrie, helped to provide new research opportunities in Toronto. Opportunities in Toronto had been opened up because of a prominent recruiting drive that had been masterminded by Murray Hunt. Murray, an early worker in gingival collagenase, was an academic with long-range vision who realized that to improve the Faculty of Dentistry at Toronto, more researchers with different types of ideas were needed. Tony was one of Murray's most auspicious "idea men". Indeed, Tony's recognition that a collaborative, multi-disciplinary research group could greatly advance the study of complex research areas such as periodontal physiology and promote the teaching of graduate students still serves as a model in the dental sciences for what can be achieved with this type of approach.

The avowed purpose of the MRC Group was to study the periodontium, its tissues, and, in particular, the origin, function, and interactions of its diverse cell populations. Twenty-five years later, this research group is still extant and is now directed by Dr. Jaro Sodek, one of its original principal investigators. All of the principal investigators but Tony were non-dentally-trained PhDs. Don Brunette, a cell biologist, was recruited in particular to develop sound, reproducible methods to culture the cells of the periodontal ligament and to develop experimental systems to examine their activity, this at a time when approaches to the culture of cells from periodontal tissues were primitive in the extreme and not routinely conducted. Don quickly accomplished the desired goals (Brunette *et al.*, 1976) and in so doing, provided the foundation for numerous collaborative endeavors among Tony, the other principal investigators (including Johan Heersche, a bone cell physiologist), and the stream of post-doctoral fellows and graduate students who passed through the group's laboratories.

The MRC Group in Periodontal Physiology

In general, the experiments conducted at the MRC Group led to an understanding of what made cells from periodontal tissues special and also proved of interest to the broader biological community, beyond that of the dental sciences. This is particularly evident when we examine in what types of journals Tony and his colleagues were publishing their papers. Although some of the more dentally related research was published in dental specialty journals, much of the work came out in general-interest science journals, a testimony to the depth of the investigations and their ability to interest a broad audience. Further, it was notable that some of these systems could be established to replicate various *in vivo* phenomena such as tooth eruption and regeneration of periodontal ligament and dento-gingival fibers. Examples of *in vitro* models from early studies involved a mixed epithelial and fibroblast culture system in which it was shown that the fibroblasts and epithelial cell rests of Malassez from periodontal ligaments recapitulate some of the behavior that characterized these cells *in vivo* (Brunette *et al.*, 1977). In one particularly fascinating study with Cathy Birek, fluid-containing domes were developed by the epithelial cells, providing a culture model of the cyst-like behavior of oral epithelial cells (Birek *et al.*, 1982). In another series of experiments, Tony, Carl Bellows, and Jane Aubin used an *in vitro* model for cell traction that had been described by Bell to compare the behavior of periodontal ligament cells with that of connective tissue cells from other tissues and to study the remodeling of extracellular matrices by cell tension. They demonstrated that periodontal ligament cells behave differently from the other connective tissue cells that were tested in the system, and provided direct proof for the presence in periodontal ligament of fibroblast-derived tractional forces (Bellows *et al.*, 1980, 1981, 1982). Such forces had been suggested as possibly contributing to the mesial drift of teeth *in vivo*. In a similar vein, he and Sandu Pitaru collaborated to show that gingival fibroblasts seeded *in vitro*

in a space between two slices of tooth became oriented between the two teeth and produced fibers reminiscent of those of the transseptal and dento-gingival fibers (Pitaru and Melcher, 1987). These studies suggested that, in the regeneration of the periodontium *in vivo*, traction of cells leads to their orientation and to the subsequent orientation of the products of fibrillogenesis.

Another approach was taken on the initiative of one of Tony's graduate students, the late Costas Maniatopoulos. The development of the new system was based on work that had been done by Carl Bellows and Jane Aubin on bone cells obtained from embryonic calvaria. Costas and Tony developed conditions in which cells obtained from adult bone marrow could form mineralized bone nodules *in vitro* (Maniatopoulos *et al.*, 1988), a method that is still widely used for studies of mineralization and the identification of osteogenic progenitor cells. This work also provided an experimental system that permitted investigation of a question that arose out of experiments conducted previously by many investigators on re-implantation of teeth and an *in vivo* experiment done some years earlier (Melcher, 1976). The question harkened back to Tony's interests in cellular domains: Do cells of the periodontal ligament play a role in maintaining the volume of the cell domain of the periodontal ligament? The investigation showed that periodontal ligament cells likely play an important regulatory role, since osteogenesis by cells obtained from rat bone marrow was inhibited by prostaglandin E2 secreted by the periodontal ligament cells with which they were co-cultured (Ogiso *et al.*, 1991). So while the periodontal ligament may contain progenitors of osteoblasts, the ability of these osteogenic cells to secrete mineralized matrix is kept in check by anti-osteogenic factors released by periodontal ligament fibroblasts. Without these anti-osteogenic factors, the alveolar bone grows into the periodontal ligament space, and the root of the tooth becomes ankylosed to the bone.

Primacy of the periodontal ligament in regeneration of the periodontium

A fascinating feature of the periodontium is the close apposition of multiple types of soft and mineralizing connective tissues and its covering by various investing layers of epithelium. The architectural complexity of the periodontium is, of course, one of the reasons why it is so difficult to regenerate this tissue following either trauma or periodontitis. Over the course of his scientific work, Tony's focus moved through several compartments in the periodontium, from bone to gingiva to periodontal ligament and back again to bone. Following a series of absolutely intriguing experiments on the contribution of the periodontal ligament to healing wounds (*e.g.*, Gould *et al.*, 1980) and the observation that the periodontal ligament appeared to inhibit osteogenesis (Melcher, 1970; Fig. 2), Tony developed a general theory on the central role of the periodontal ligament in periodontal regeneration. The ideas that have arisen from this theory have made it one of his most widely quoted papers (Melcher, 1976) and to this day it still is quoted by workers in the periodontal regeneration field. In essence, Tony observed that the progenitor cells for cementum, bone, and periodontal ligament fibroblasts were all contained right in this tissue. Consequently, it made sense that if periodontal regeneration were to be achieved, then the periodontal ligament would have to be regenerated first. With this in mind, Tony, Don Brunette, and Tim Gould applied this exact idea to an investigation that sought to identify the location of cells in the periodontal ligament that divide after wounding. They placed a small piece of mylar film over the periodontal defect in an effort to prevent connective tissue cells originating from outside of the periodontium from entering the wound—that is, from entering into the alveolar bone cell "domain" (Gould *et al.*, 1980).

This belief in the integrity of cellular domains subsequently formed the basis of the ideas developed by Nyman and Karring on guided tissue regeneration. In this approach, exclusion of soft fibrous connective tissue cells is thought to enhance the recolonization of periodontal wounds by cells from the periodontal ligament and alveolar bone. Although the biological basis of guided tissue regeneration is still incompletely understood, the "roots" of this idea can be easily traced to Tony's early experimental work and theory.

In a highly quoted paper written with Gerry Boyko and Don Brunette (Boyko *et al.*, 1981), Tony described how teeth bearing cultured periodontal ligament cells transplanted into bony sites could generate a new, functionally oriented periodontal ligament. In contrast, the transplantation of teeth bearing cultured gingival cells did not lead to reformation of the periodontal ligament. This experiment provided very interesting proof to show that the periodontal ligament was indeed of central importance to the regeneration of the periodontium. The experiment was also "light years" ahead of its time, since only now are papers starting to appear that consider the use of transplanted cells

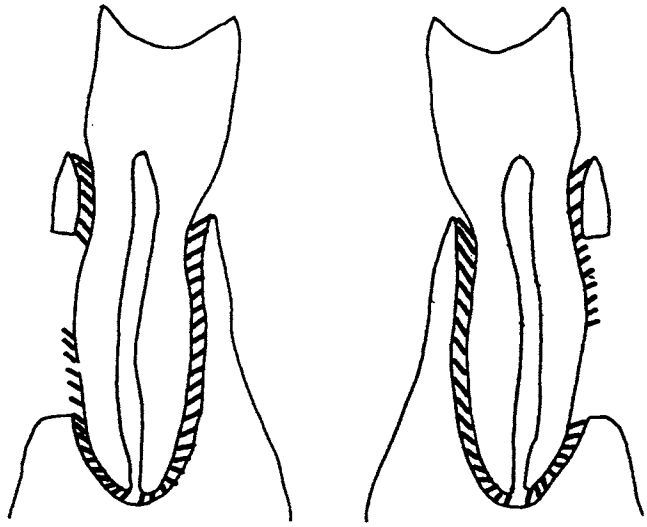

Figure 2. A diagram of the periodontal ligament extirpation model developed by Tony to study the influence of the periodontal ligament on osteogenesis (from Melcher, 1970).

Figure 3. At a conference in honor of Tony's retirement from the University of Toronto. Thirty colleagues, students, and post-doctoral fellows gathered to present their own work since graduating from the "Tony Melcher School of Research".

for reconstitution of lost periodontal tissues.

Taken together, these diverse studies have helped to advance the field in three important ways: First, they have improved our understanding of the origin, the function, and the regulation of individual cell populations in the periodontium; second, the development of simplified culture systems facilitated experiments that showed which specific cellular components were responsible for certain *in vivo* functions of the periodontium; and third, they have paved the way to the production of purified populations of cells from gingiva, bone, and periodontal ligament. This has now opened up the possibility of cell transplantation into a wound defect for the purpose of periodontal regeneration. So, from simply filling holes in alveolar bone with pieces of anorganic bone or other bone grafts, we can now approach the challenge of periodontal regeneration with the most powerful biological engines—the cells of the periodontium.

Epilogue

Tony became Vice-Provost of the University of Toronto for Health Research in 1988 and was totally involved in administrative work until his retirement from the university in 1992. Since then, he has continued along the collaborative route, participating in a multidisciplinary study rather different from that which had gone before. He used computed tomography to reconstruct the jaws, dentition, and pathological features of a 2800-year-old Egyptian mummy! (Melcher *et al.*, 1997) So, from the periodontium to the world of archaeological reconstruction and the demonstration that dental disease 800 years BCE was a potentially fatal condition, Tony has moved on. Permeating all of his work have been an excitement and a view different from what was currently held. It is his ability to ask interesting questions and then to identify the most promising techniques for answering them that put him and his co-workers at the forefront of periodontal regeneration for so many years. In a symposium held in his honor in 1992, 32 former students, post-doctoral fellows, and co-workers from eight countries assembled in Toronto to give presentations on their current work (Fig. 3). Each of these speakers paid Tony the ultimate tribute by showing where his guidance and scientific leadership had led them in their own research. It was a fitting testimonial to his scientific life.

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