Panel Discussion: Lessons Learned and Future Directions

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The 2013 San Francisco symposium has brought together many of the leading researchers in the pulp biology and regenerative endodontics areas, helping to define the current state of the art in the field and, importantly, identifying critical questions still needing to be addressed for the goal of clinical translation of regenerative endodontics to become a reality in everyday practice. At the conclusion of the symposium, the symposium speakers and participants came together for a podium discussion to further explore some of these critical questions and for many of the speakers to pose what they regard as the "burning questions" in their specific areas. This article attempts to briefly summarize these discussions and to highlight areas requiring further research, which may be valuable to those researching in this field. The scope of the discussions can be essentially categorized under 4 broad headings (Fig. 1), which will provide the basis for this summary.

Cells

A fundamental requirement for pulp regeneration is the recruitment of stem/progenitor cells to the site of tissue injury before their differentiation and subsequent participation in the genesis and remodeling of the tissue. Although much of the focus has been on the cells involved in odontoblastlike cell differentiation for the secretion of new dentin to regenerate the hard tissue structure of the tooth, this must be accompanied by differentiation of other cell types participating in important parallel biological processes, such as angiogenesis, for overall regeneration of tissue architecture.

Our understanding of physiological odontoblast differentiation is based on the tightly regulated events of tooth development in which the temporospatial control of the emergence of odontoblasts at sites of dentin formation is both very predictable and reproducible. However, in regenerative situations, we move from physiological to pathological processes. Probably the most significant consequence of this is that the tight temporospatial control of events observed during tooth development is largely lost along with the predictability and reproducibility of cellular events. As a consequence, a broad spectrum of tissue events will often be observed in the pulp after injury with outcomes ranging from repair to regeneration. Although it is possible to become buried in semantics and to debate how we describe the responses noted during pulpal wound healing, it is essential that we consider how closely these responses mirror physiological processes. This clearly has consequences for how new therapeutic protocols are developed as clinical translation progresses.

The identification of specific stem cell populations in the pulp has provided a major stimulus to understanding pulp regeneration. However, increasingly, both in this and other fields of tissue regeneration and engineering, there is recognition that the expression of surface markers used for the identification of these mesenchymal stem cell (MSC) populations may not be quite as reliable as initially predicted. It seems probable that the niche in which these cells reside will influence their surface marker profile and may partly account for some of the profile differences between circulating and tissue-resident MSCs. During the symposium, the importance of the perivascular niche for these cells in the pulp was highlighted, but our understanding of the precise nature of this and other niches remains in its infancy. An improved understanding of the stem cell niche may offer opportunities to protect or preserve stem cell populations in the pulp, to more effectively recruit these cells to sites of tissue injury, and to possibly modulate the niche for therapeutic advantage.

The recognition of stem cell populations resident in the pulp has focused much attention on local tissue events, but data presented suggest that circulating MSCs may also migrate to the pulp after injury. Thus, new therapeutic strategies should also

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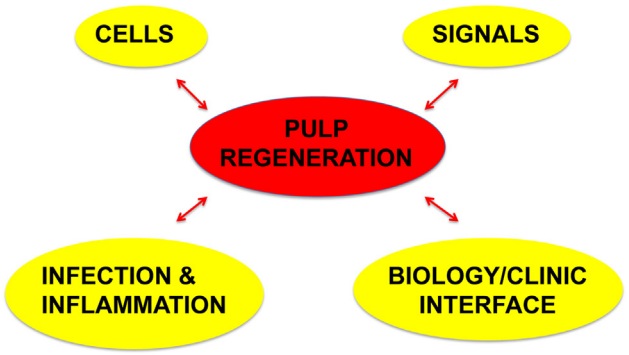

Figure 1. Podium discussion areas.

consider exploitation of a wider potential pool of cells for regeneration. This also emphasizes the importance of a well-developed vasculature in the pulp. Angiogenesis will be important not only for nutrient supply during regeneration of the tissue but also, potentially, for stem cell recruitment. There is still much to learn about angiogenesis in the pulp, both in physiological and pathological conditions. The identification of the cell populations responsible for pulp angiogenesis represents a key step in developing new regenerative strategies and the recognition that stem cell populations, such as SHED cells, can give rise to both odontoblastlike and endothelial-like cells, reflecting the complexity of understanding regenerative events.

A major consideration in the development of new regenerative strategies is whether to adopt cell-based or cell-free protocols. The ability to introduce specific cell populations with defined properties to the injured pulp is a significant advantage for cell-based protocols. However, this also brings significant challenges in isolating stem cell populations of sufficient efficacy, purity, and quality for clinical transplantation that meet all of the safety and regulatory requirements. The use of cell-free protocols is attractive in terms of avoiding some of these challenges and exploiting endogenous stem cells either resident in the pulp or recruited from outside the pulp but offers different challenges in terms of requiring stem cell recruitment step(s) to be a part of any regenerative protocol and, possibly, the protocol being less predictable in its outcomes. Nevertheless, at this stage, there does not appear to be any clear indication as to which protocol will likely yield optimal clinical outcomes, and there will be considerable merit in pursuing both protocol approaches as we endeavour to progress the clinical translation of pulp regeneration.

Burning questions posed by speakers for this session of the symposium included the following:

Michel Goldberg: Are the stem cells located in the apical niche of the pulp playing a key role in pulp regeneration?

Imad About: Pulp progenitor cells are defined as resting cells. After infection and injury, these cells require activation and differentiation signals that are produced from the local environment at the injury site. These signals allow not only dentin regeneration but also angiogenesis and pulp regeneration. Do we need to transplant only stem cells in order to obtain pulp regeneration or a heterogeneous population of pulp cells enriched with stem cells?

Misako Nakashima: How do we address the challenges involved in the preparation and quality control of clinical grade dental pulp stem cells, which are safe and show efficacy?

Kerstin Galler: Can the concept of pulpotomy be extended to permanent teeth with complete root formation? Will resident stem cells in the remaining tissue and endogenous growth factors from the dentin matrix be sufficient for pulp regeneration (ie, are cell-free protocols feasible for clinical success, especially without introduction of exogenous signaling molecules)?

Signals

Signaling of cellular events is fundamental to driving the different processes resulting in pulp regeneration, and much of our knowledge base is derived from study of cellular signaling during physiological tooth development. However, although these signaling events are tightly regulated during tooth development, such regulation is much less coordinated in pulp regeneration with consequent effects on tissue outcomes. Tissue events are further exacerbated by the inflammatory and immune defense responses taking place to overcome the challenges of infection within the pulp environment as regenerative processes are initiated.

Of fundamental importance is understanding how the cell niche/extracellular environment in the pulp contributes to cell fate and phenotype. The identification of specific stem cell populations in the pulp, albeit showing similarities to mesenchymal stem cells, implies that the local environment of the pulp is responsible for signaling the acquisition of these specific characteristics of pulp stem cells. Whether these stem cells are resident populations arising during tooth development or recruited from sources outside the tooth after development, they will be exposed to similar influences from the pulp environment. However, the pulp environment is complex and likely to show many variations at different sites and under different tissue conditions of either health or disease. At 1 level, the stem cell niche, which may be perivascular in origin for many of the stem cells in pulp, will be important in maintaining the "stemness" of these cells. Our understanding of the pulp stem cell niche is still very limited although it may be possible to extrapolate from findings for stem cells at other sites in the body. Further characterization of the cell niche is important to understanding how we may manipulate the behavior and fate of these cells. However, it is important to try and model these cell-matrix interactions under conditions resembling those *in vivo* because much of the work on pulp stem cells has been performed *in vitro* with culture of these cells on plastic surfaces, which show little similarity to *in vivo* conditions.

Once the pulp stem cells leave the influence of their niches, which maintain their stemness, they will be exposed to a variety of signals including those responsible for directing their migration, proliferation, and differentiation (ie, cellular events largely associated with repair, regeneration, and wound healing). Considerable progress has been made toward characterizing the signaling involved in these events although far less is known of the control and regulation of this signaling. This is perhaps in part caused by the varied sources of the signaling molecules, which may be released into the cellular environment as a result of the pathological events associated with the injury to a tissue and subsequent defense responses. It is now clear that dentin matrix is not simply an inert, structural matrix and contains a reservoir of growth factors and other bioactive molecules sequestered or "fossilized" within the mineralized matrix. Demineralization of the matrix during caries and some restorative procedures can locally release these bioactive molecules at sites of injury where they will contribute to signaling of subsequent tissue events. Although the potential of these molecules to direct regenerative events is now recognized, there is still much to learn as to how this signaling can be harnessed for the most effective outcomes in the clinical situation. Pulp cells and their extracellular matrices, as well as some defense cells, will also likely be a source of some of the signaling molecules involved in regeneration. The relative contributions of these various sources and their importance at different stages of the regenerative process require further study.