The *Conundrum* of Human Osteoinduction: Is the Bone Induction Principle Failing Clinical Translation?

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Experimental work starting in the late 19th and into the early 20th century discovered that bone tissue (and indeed other tissues) contains diffusible substances that trigger the induction of bone formation.[1–3] Levander proved that alcoholic extracts of bone induce the formation of bone when implanted in heterotopic extraskeletal sites, (where there is no bone).[1] The hypothesis of putative substances or "*morphogens*", (first described by Turing as "*forms generating substances*"),[4] was a fundamental concept preceding several extraordinary experimentalists poised to dissect the rules of tissue induction and morphogenesis.[4] Amongst others, Urist and Reddi persevered to identify the putative osteogenic activity present within the extracellular matrix of bone, dentine and other matrices including the kidney and the uroepithelium.[5–7]

The classic work of Urist identified the critical role of acid demineralization of the bone matrix to predictably induce new bone formation in extra-skeletal heterotopic sites of rodents and lagomorphs, and proposed the term bone morphogenetic protein complex within the bone matrix initiating *de novo* bone formation by induction.[6] The therapeutic exploitation of the "bone induction principle"[8] was immediately seized upon for the treatment of tibial non-union in humans using demineralized bone matrices.[5,8] Continuous research across the globe finally yielded protein purification to homogeneity with amino-acid sequencing resulting in molecular cloning of a series of human bone morphogenetic proteins (hBMPs), members of the transforming growth factor-β (TGF-β) supergene family.[9,10] After the incisive work of Wozney and Özkaynak in 1988 and 1990, respectively,[9,10] cloning was followed by the production of recombinant human BMP-2 and BMP-7. The biological activity of the recombinant human morphogens was confirmed by outstanding data from several animal studies, including non-human primates' species.[11]

Whilst occasional reports appeared that validated the clinical efficacy of BMPs to regenerate bone in human patients,[12,13] all too often critical examination of published radiographic images revealed the failure to achieve clinically significant osteoinduction and bone regeneration comparable to autologous bone auto transplants. The next three decades of therapeutic use enforced the sobering realisation that translation of animal trial results to humans was all too unpredictable.[14–25] The question this raises is what are the differences, and why are there differences in biological response to osteoinductive morphogens between most mammals (and even non-human primates) and *Homo sapiens*, possibly for molecular and physiological discrepancies in regulatory complexity between species.[26] We are not in the position to answer these questions at present; however, it places researchers and clinicians

in this field in an uncomfortable position – how do we interpret animal trial data knowing that its usefulness is limited? At present, the only answer can be with great caution.

Knowing as we now do that the use of a single morphogen delivered by collagenous substrata routinely fails to match autologous bone in clinical use, a critical reappraisal of future osteoinductive strategies in humans is required. Using the autologous bone graft as a sensible blueprint we must seek to replicate the multiple constituents that confer on a bone graft the capacity to regenerate bone post transplantation. With this in mind, it is necessary to consider the cellular, mineral and morphogen constituents of a bone graft.

Selection of the most appropriate osteoinductive morphogen or morphogen combination is a fundamental consideration. The clinical use of either hBMP-2 or hBMP-7 *solo* has so far yielded results that are too unpredictable to allow clinicians to use them with any degree of confidence. It behoves to reconsider the biological relevance of the previously dismissed "*apparent redundancy*", i.e. why are there several members of the TGF-β superfamily demonstrating osteoinductive capabilities.[11] Whilst it may indeed be a protective redundancy, it is more likely that synergistic activity of multiple morphogens deployed at critical spatial and temporal junctures will optimize bone tissue regeneration. Morphogen choice should no longer be based on expediency but on biological imperatives.

Systematic preclinical studies in the Chacma baboon *Papio ursinus* showed that the three recombinant human mammalian transforming growth factor-βs induce substantial amounts of bone formation when implanted heterotopically in the *rectus abdominis* muscle.[11,27–29] The hTGF-β_3 isoform engineers hyper cellular bone organoids with rapid induction of mineralized bone and osteoid, the latter populated by highly secreting contiguous osteoblasts.[27–29] Gene expression pathways by qRT-PCR show that the induction of bone formation is *via* several profiled *BMPs* expressed following implantation of hTGF-β_3.[27–29] This downstream expression of *BMPs* elicited by hTGF-β_3 may escape the antagonistic activity of Noggin, whereas direct implantation of high doses (often several mg) of hBMPs activates the Noggin antagonist pathway and may explain the limited effectiveness of hBMPs in clinical contexts.[27–29]

Synergistic binary applications or single relatively high doses of hTGF-β_3 have shown that hTGF-β_3 induces bone by expressing a variety of *bone morphogenetic proteins* (*BMPs*). Tissue induction thus invocated singly by hTGF-β_3 recapitulates the synergistic induction of bone formation by binary applications of hTGF-β_1 and -β_3 with hBMP-7.[30] The induction of bone formation could be profoundly enhanced by binary applications of a recombinant hBMP with relatively low dose of hTGF-β_1 or -β_3 with a ratio by weight of 20:1.[11,30] Molecularly, the rapid induction of bone formation by binary applications of hBMP-7 and hTGF-β_3 or by hTGF-β_3 applied singly resides in the up-regulation of selected genes involved in tissue induction and morphogenesis. Genes include *Osteocalcin*, *RUNX-2*, *BMP-7*, *TGF-β_1* and *-β_3*, with however down regulation of *TGF-β_2*.[30] Synergistic binary applications also induce the morphogenesis of rudimentary embryonic growth plates indicating that the "memory" of developmental events in embryo can be re-deployed post-natally by the application of morphogens' combinations.

The biological acceptance of the inductive activity of a single recombinant human protein above the natural *milieu* and *equilibrium* of the extracellular matrix of bone containing several pleiotropic naturally derived morphogens attached to the mineralized collagenous matrix of bone has been the fundamental error of the biotechnology industry developing

recombinant hBMPs for translation in clinical contexts.[31] Eager clinician scientists embraced the powerful biological activity of a single recombinant hBMP with no proper efficacy studies beyond *in vitro* and *in vivo* rodent' models.[18,31]

A final, and as it turned out, fundamental error of biotechnology companies, was to seek regulatory approval for doses much higher than those tested in pre-clinical animal studies justified by the vague *rationale* that higher doses were needed in clinical contexts.[31] It turned out that even massive doses of several milligrams proteins per gram of carrier, (aside serious collateral effects), were needed to yield insufficient regeneration often inferior to autogenous bone grafts.[14,19–25]

Morphogens' delivery matrices are enforced upon physicians by commercial dictates that have proven in humans to be inadequate. Delivery system choices are bewildering but suffice to say that the material characteristics that are required are compression resistance, to bind and desorb morphogen at appropriate rates and resorb readily. Whilst allografts and some synthetic ceramics have shown the characteristics of spontaneous osteoinductivity the phenomenon is inadequate for therapeutic use although may exploited for synergy between delivery and morphogen. It must be conceded that at present, the insoluble collagenous bone matrix or demineralized bone matrix remains the most appropriate choice for delivery, and future investigations may focus on combining demineralized bone matrices with polymers that will allow the manufacture of customisable shapes.

What cellular populations are required can be determined by the careful study of the cellular populations of bone grafts. Once we know these facts, the next stage is to determine where and how are these cells to be obtained? The stem cell *niche* has become yet another medical *plat du jour* promising to solve many of the regenerative challenges of medicine. Harvesting, isolating and expanding a cellular population is expensive, time consuming and at present of little clinical importance. Returning to the constituents of an autologous bone graft as a waypoint it seems that the most pragmatic approach to obtain relevant osteogenic cellular populations via low morbidity bone aspirates from one of several donor sites.[32]

In his optimistic classic Editorial Comment "The reality of a nebulous enigmatic myth",[33] Marshall Urist stated that pre-clinical and clinical research on the bone induction principle[8] "are bound to dispel the myth and appreciate the reality of bone induction for the benefit of patients with crippling diseases of the bone and joints". Fifty years later, reading that several tens of milligrams of hBMPs are needed to induce an uninspiring bone volume in human patients the Bone Research Laboratory, (not in Los Angeles but in Johannesburg), still wrestles with this nebulous enigma.

We have repeatedly posed the challenge to molecular biologists, clinicians, tissue engineers and regulators alike to encourage and facilitate the exploration of a novel approach to therapeutic bone regeneration. We are convinced it will be a vanguard that will deliver the goal that surgeons and patients aspire to – a low morbidity, reliable technique for the reconstruction of osseous defects of any size.

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