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# Healing with RNA

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Readers of *Injury* spend a considerable portion of their professional lives mitigating the effects of trauma on their patients' organs. In many disciplines, such as orthopaedics, interventions have traditionally been surgical. They have reached high levels of sophistication. Open reduction and internal fixation, for example, has largely replaced splinting and has led to the development of advanced technologies for stabilizing fractures with customised plates and intramedullary nails (1). Progress in these endeavours required close collaborations between surgeons, metallurgists and engineers.

It is widely thought that the next quantum advance in traumatology will come from the realm of biology. In particular, tissue engineering and regenerative medicine (TERM) promise to regrow tissues and organs with unparalleled authenticity, such that the repaired organ will be indistinguishable from its native, uninjured counterpart. A key component of this process involves guiding the behaviour of the cells that will generate the new tissues.

Under physiological conditions, cell behaviour is regulated by numerous physical, chemical and biological signals. Of these, various proteins that function as morphogens and growth factors have received the most attention in terms of clinical application. Indeed, recombinant bone morphogenetic protein-2 (BMP-2) is approved by the FDA as a component of Infuse® for certain indications where it is necessary to grow bone. However, its clinical efficacy has been disappointing and its use accompanied by adverse side effects (2). Few other recombinant growth factors have made it to market for regenerating the musculoskeletal system.

Delivery problems have been commonly blamed for the disappointing clinical efficacy of recombinant growth factors. In particular, it is difficult to deliver the required doses of a specific growth factor at the site of injury in a sustained manner. In response to this, there is a considerable body of research into the use of smart scaffolds as implantable

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delivery devices (3). Local gene delivery is another promising area of investigation, with the advantage that the protein of interest is produced nascently with authentic post-translational modifications (4). Significant pre-clinical progress has been made, but the disadvantage of genetic delivery is the cost and regulatory complexity of clinical translation.

RNA, the intermediary between a gene and its cognate protein, may offer the best of both worlds. After transfection into a cell, messenger RNA (mRNA) will almost immediately begin to express its encoded protein in the cytoplasm without the need to translocate to the nucleus. The protein will be expressed for a period of time, after which the RNA is degraded by innate physiological processes that leave no residue. Because the RNA remains cytoplasmic, there is no danger of insertional mutagenesis or other genetic damage, unlike the case with DNA therapeutics.

These theoretical advantages of mRNA, while appreciated for a long time, could not be immediately exploited. When added to cells, mRNA is cytotoxic, inflammatory and, in most cases, it has a short half-life. These limitations have been overcome by the development of chemically modified RNA (cmRNA) that is more stable, less inflammatory and less toxic. The alterations that achieve this include the chemical modification of certain pyrimidine residues and manipulation of untranslated regions of the RNA, including certain open reading frames and the polyadenosine tail.

Pre-clinical data suggest that cmRNA will be of utility in bone healing (5) and tendon repair (6). In addition, cmRNA encoding VEGF-A recently demonstrated angiogenic potential and has advanced to a phase I clinical trial in patients with diabetes (7). More generally, it is being explored as a means of vaccination against diverse tumours (8) and viruses (9).

There are additional ways in which RNA can be used therapeutically. Whereas cmRNA is used to deliver a specific protein, other species of RNA inhibit gene expression through a process of RNA interference (10). MicroRNA (miRNA), small interfering RNA (siRNA), short hairpin RNA (shRNA) and long, non-coding RNA (lncRNA) are of especial interest in this regard. The FDA recently approved the first RNA drug based on RNA interference for the treatment of a rare form of amyloidosis.

In the musculoskeletal field, there are numerous non-coding RNA molecules associated with regeneration or its inhibition. Kelch *et al.* (11), for instance, identified miRNA-100 which inhibits osteogenesis and is correlated with reduced bone mineral density. When this miRNA is blocked by antagomirs, inhibition of its target BMP-R2 mRNA is relieved, expression of collagen I and runX2 is restored and osteoblast function recovered. In a related application, anti-sense RNA to miRNA-214 delivered on a silk scaffold improves the differentiation of human mesenchymal stem cells (MSCs) into osteoblasts (12). Collectively, the literature suggests an important role for miRNA in fracture healing (13). Two miRNAs, miR-145 and miR-140, are important in the differentiation of MSCs into chondrocytes (14), and a number of miRNAs are important for cartilage homeostasis (15). Several miRNAs are associated with tenogenesis (16).

RNA therapeutics have enormous momentum with considerable potential application in the world of TERM. In the near to mid-term, traumatologists may well find themselves using RNA-based formulations to regenerate tissue and restore function to their patients.

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