J Mol Med (2000) 78:303–311
DOI 10.1007/s001090000118

REVIEW

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Tissue engineering via local gene delivery

Received: 1 February 2000 / Accepted: 8 May 2000 / Published online: 29 June 2000

Abstract The first goal of this review is to describe a local plasmid gene transfer technology known as the gene activated matrix (GAM). GAM was the first gene therapy designed specifically for tissue engineering applications, and the mechanism of action of plasmid gene transfer is closely tied to the normal sequence of events associated with wound healing. The normal sequence of wound healing events is stereotyped for most tissues, and one assumption has been that GAM could serve as a platform technology for local gene delivery in various tissues and organs. This hypothesis essentially has been proved: animal studies over the past 6 years have established that plasmid genes can be delivered to acutely injured tendon, ligament, bone, muscle, skin, and nerve. The second goal of the review is to describe the most likely "first use" of the technology in man, namely, treatment of osteoporotic hip fracture in the elderly. Although not universally appreciated, interest in osteoporotic fracture should grow because of epidemiological, surgical, and societal considerations. These considerations, plus the unmet clinical need associated with the current standard of fracture care, justify efforts to develop novel therapies for bone regeneration and repair in the elderly.

Key words Wound healing · Granulation tissue · Growth factor · Gene transfer · Gene activated matrix

Abbreviations *GAM*: Gene activated matrix · *PTH*: Parathyroid hormone

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Clinical aspects of wound healing

All told, the annual cost of injured or failed human tissues and organs runs into billions of dollars and is associated with significant loss in productive quality of life [1]. Certainly, clinical wound healing is not free of complications: the capacity for robust regeneration in most vertebrates may be limited to those tissues (e.g., liver, bone, and skeletal muscle) in which regeneration partially recapitulates embryonic differentiation from multipotential stem cells. A general approach of regenerative biology is to identify the cellular and molecular differences that distinguish tissue embryogenesis from wound repair (scarring) and then to recreate an embryonic (regenerative) environment in the injured adult tissue. (Identification and characterization of these environments may one day form the basis for rational product development.) Limited success in stimulating the regeneration of mammalian bone, skin, blood vessel, and spinal cord has been achieved by bridging lesions with artificial or natural biomaterial scaffolds that promote cell migration, proliferation, and differentiation [2]. The functional integrity of damaged tissues can also be restored today with replacement devices and organ transplants, but these modalities are limited in availability and effectiveness and are associated with significant medical sequelae.

The emerging discipline of tissue engineering has presented an alternative strategy based on transplantation of constructs consisting of endogenous stem/progenitor cells grown ex vivo within predesigned matrix scaffolds [3]. The scaffold eventually is resorbed, leaving only

transplanted cells and the stroma that they produce in the body. (A final structure sometimes referred to as a neo-organ.) Eventually it should be possible to engineer large, complex organs (e.g., liver, kidney, bladder, and gut) using this strategy. Off-the-shelf tissue engineering products (that contain cells) are not currently available; however, and it generally takes weeks to generate a neo-organ ex vivo. Therefore the neo-organ strategy generally is available only for chronic tissue injury applications, for example, replacement of defective hyaline cartilage due to osteoarthritis.

Although they are rational drug targets, the clinical promise of cytokines and growth factors for acute tissue injury applications has yet to be achieved despite intense effort for more than a decade [4]. The major concern with systemic delivery is that too little of the cytokine drug is delivered to the diseased tissue target to be effective, while too much of the cytokine is delivered to bystander tissues (expressing the relevant cell surface receptor) to be safe. An extensive database exists on this point, i.e., there are hundreds of articles that provide direct evidence of cytokine toxicity. Moreover, this literature includes (but is not limited to) tissue repair and immune response cytokines (e.g., [5]). In response to this challenge, a second tissue engineering strategy involves the sustained, local delivery of recombinant cytokines and growth factors directly into wounds so as to coordinate an appropriate cellular regeneration/repair response [6]. However, several barriers to effective and safe local delivery in vivo have been identified [7]. These barriers are related to pharmacokinetics (recombinant growth factors are in many instances too short-lived to be effective) and manufacturing (recombinant growth factors are costly to produce). A third barrier is related to formulation. Sustained-release systems capable of local recombinant cytokine drug delivery to specific body sites for prolonged times should improve potency and may offer a lower risk of toxicity [6, 7]. With few exceptions (e.g., [8, 9, 10]), however, it has been difficult to maintain full bioactivity following recombinant cytokine and growth factor incorporation into controlled delivery systems. Moreover, high-dose local delivery is associated with both local and systemic toxicity, the latter presumably through cytokine diffusion from the wound bed into the bloodstream, i.e., dose dumping. Together, these barriers help describe the relatively narrow therapeutic window of many recombinant cytokines and growth factors in vivo and suggest a possible explanation for disappointing human clinical trial results.

Gene activated matrix technology

We have proposed that these barriers to delivery could be overcome if cytokines and growth factors could be delivered not as recombinant proteins but as plasmid genes [11]. Following gene transfer, the recombinant cytokine could (in theory) be expressed in situ by endogenous wound healing cells – in small amounts but for a prolonged period of time – leading to reproducible tissue regeneration. Plasmid DNA is well known to possess a stable, flexible chemistry that is compatible with established polymer-based drug delivery systems. Plasmid diffusion from the delivery site should not in theory cause systemic toxicity because of the high efficiency of DNA catabolism in the bloodstream [12]. Finally, plasmid DNA is economical and relatively simple to manufacture [13] and is nontoxic to tissues if manufactured in an appropriate manner.

To explore this proposal, a local gene delivery system for tissue engineering applications, referred to as the gene activated matrix (GAM) [11], was developed. At its most basic, a GAM consists of two ingredients: plasmid DNA and a biodegradable structural matrix carrier. GAMs may take several forms (e.g., a lyophile implant or sponge, an injectable gel or paste, and a medical device coating) that can all be manufactured as off-the-shelf products for direct placement into an acute wound bed.

Wound healing in mammals is a highly evolved process [14]. The destruction associated with tissue injury engenders a concerted response that initially focuses on controlling hemorrhage. Repair then begins with the formation of granulation tissue and ends with either scar formation (repair) or tissue regeneration. (Granulation tissue consists of proliferating fibroblasts and capillary blood vessels that originate at the margin of the tissue wound and migrate into the wound bed.) The cells that participate in wound healing include platelets, acute inflammatory cells, macrophages, fibroblasts, endothelial cells, pericytes, and tissue-specific progenitor cells. To coordinate the cellular response, cytokines and growth factors act locally through wound- and tissue-specific signal transduction cascades. An essentially identical sequence of wound healing events is observed for all tissues and organs that sustain an acute injury. Moreover, this injury-response sequence is observed following traumatic injury (e.g., bone fracture), pathology-induced injury (e.g., tissue necrosis following infection), and iatrogenic injury (e.g., tissue injury associated with surgical procedures).

The mechanism of action of GAM plasmid gene transfer is closely tied to the normal sequence of events associated with wound healing (Fig. 1). Our studies have shown that the GAM carrier serves as a scaffold that holds DNA in situ until endogenous wound healing fibroblasts arrive. Once transfected, fibroblasts in the matrix carrier act as local in vivo bioreactors, secreting plasmid-encoded proteins that augment tissue repair and regeneration. (Thus, GAMs do not follow a “drug delivery” paradigm in the traditional sense of this term.) By taking advantage of the natural propensity of granulation tissue to grow into the wound, GAMs allow for the physical (passive) targeting of repair fibroblasts and other cells for direct in vivo plasmid gene transfer. Given the near-universal nature of the wound healing sequence, passive targeting of repair cells by GAM should occur in the fresh wounds of a wide variety of tissues and organs.

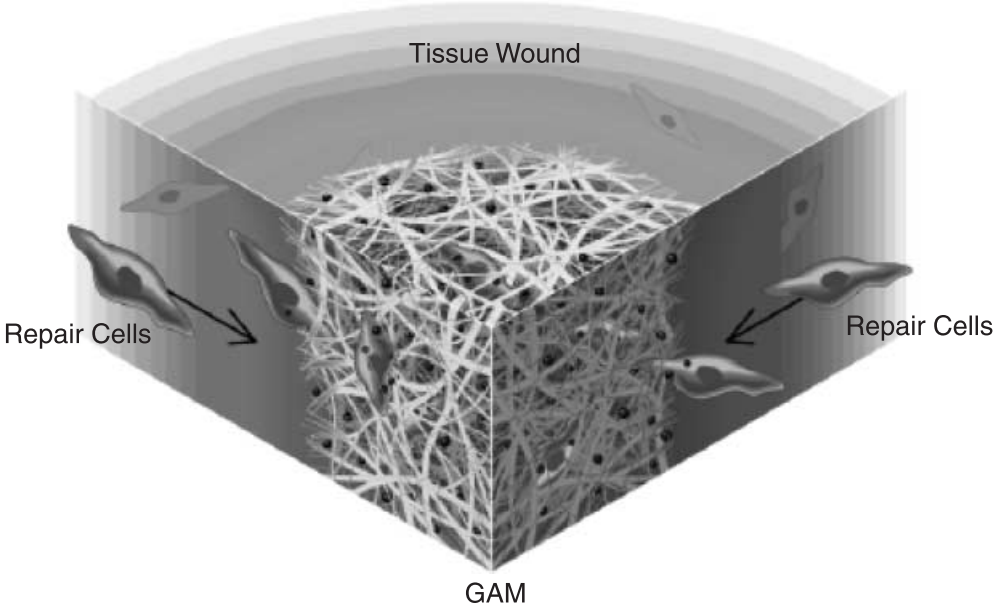

Fig. 1 The schematic figure shows a GAM implant in a fresh wound site (*inner area*). A GAM at its most basic consists of two ingredients: plasmid DNA and a structural matrix carrier. As part of the wound healing response, granulation tissue fibroblasts proliferate and migrate from viable tissue (*outer area*) surrounding the wound into the GAM. Once there, fibroblasts take up and transiently express plasmid DNA. The GAM matrix has two functions: it holds plasmid DNA in the wound site (until cells arrive), and it acts as scaffolding that promotes fibroblast ingrowth and accumulation near the DNA. While in the matrix, transfected fibroblasts act as local in vivo bioreactors, producing plasmid-encoded proteins that stimulate wound repair

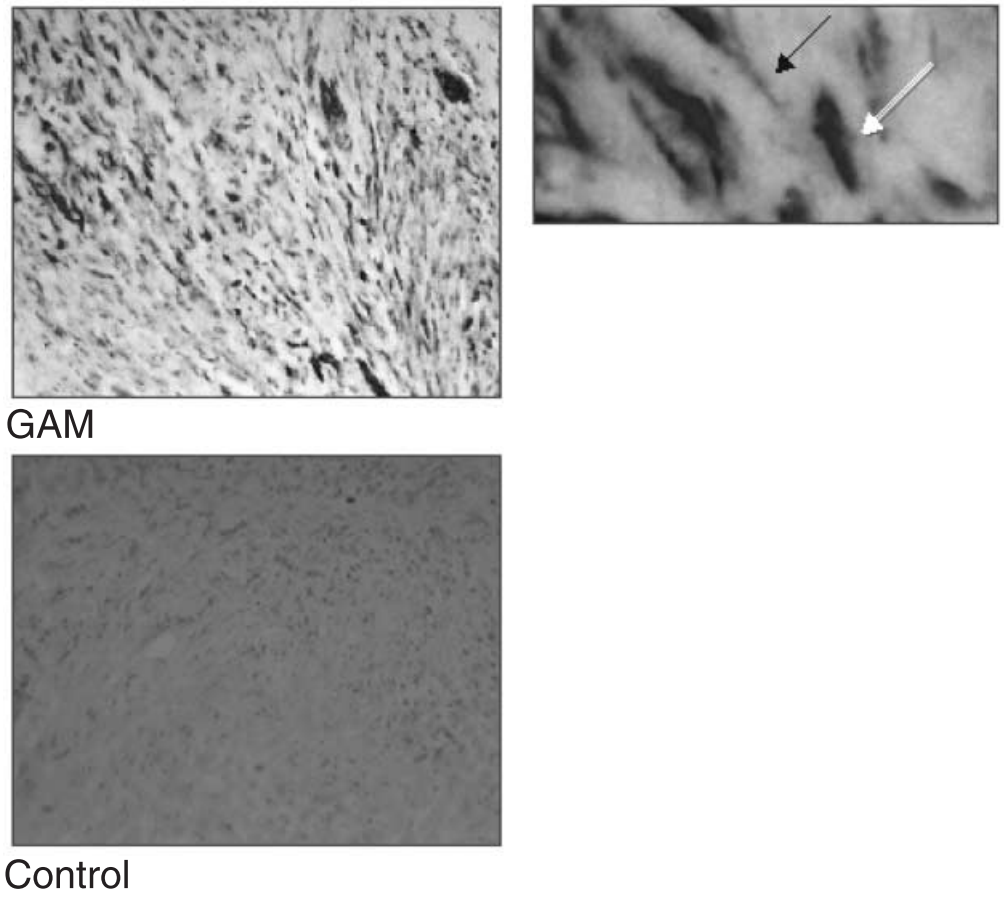

Fig. 2 Direct in vivo plasmid gene transfer into canine bone granulation tissue fibroblasts, as determined by immunohistochemistry. Upper *left* Numerous granulation tissue fibroblasts positively stained for nuclear-targeted β-galactosidase 3 weeks after GAM implant surgery. *Below* Essentially negative fibroblast staining from control defect (same dog). High magnification (*right*) clearly establishes nuclear-targeted β-galactosidase expression in fibroblast nuclei (*white arrow*), while *black arrow* points to (–) fibroblast cytoplasm. (With permission from [15])

Plasmid gene retention and expression (RNA and protein) at the site of GAM implantation is prolonged compared to recombinant protein – weeks [15] vs. hours [16], respectively – and yet still transient. Plasmid gene transfer from GAM is capable of yielding significant amounts of recombinant protein in vivo, for example, in a canine bone defect model, 1.0 mg plasmid DNA yielded picogram amounts of recombinant peptide over a 2-week period [15]. Studies with colleagues have shown that biomaterials such as collagen (lyophile sponge,

paste, and gel), hyaluronan, and alginate may be used as matrix carriers of plasmid DNA, as can synthetic materials such as poly(lactide-co-glycolide) and carboxymethylcellulose. In animal models direct GAM plasmid gene transfer to repair cells in bone [15, 17], skin [18], tendon and ligament [19], heart and skeletal muscle [20], and cranial nerve (M. Berry et al., submitted) has been reported. GAMs have also provided an unexpected level of local plasmid gene expression in vivo: we have come to expect that 30–50% of available wound healing repair cells will be transfected 3 weeks after GAM implantation, as determined by semiquantitative endpoint assays, for example, substrate utilization assays and immunohistochemistry (Fig. 2). An independent group recently reported a similar result [21]. The mechanism for this level of gene transfer efficiency is only now being explored.

To assess potency, Fang et al. [17] conducted an initial GAM feasibility study in the adult rat that involved direct plasmid gene transfer to mammalian repair cells participating in fracture repair. Implantation of GAMs containing a β-galactosidase or luciferase plasmid led to DNA uptake and functional enzyme expression by granulation tissue fibroblasts. Implantation of a GAM containing either a bone morphogenetic protein-4 plasmid or a plasmid coding for a secreted fragment of parathyroid hormone (designated here as hPTH 1-34) resulted in a biological response of new bone filling the defect. Finally, implantation of a two-plasmid GAM encoding both bone morphogenetic protein-4 and the hPTH 1-34 peptide, which act synergistically in vitro, caused new bone to form faster than with either factor alone. Bonadio et al. [15] then investigated feasibility and potency using canine bone regeneration as the endpoint in vivo. GAM implantation at sites of bone injury was associated with retention and expression of plasmid DNA for at least 6 weeks. To regenerate bone, GAM implants were again formulated with a plasmid gene encoding hPTH 1-34 peptide in a collagen sponge. The investigators found that local hPTH 1-34 expression induced the growth of centimeters of normal new bone in a safe, stable, and reproducible manner that was both dose- and time-dependent (Fig. 3).

Beyond the effort to regenerate bone, Shea et al. [18] investigated the feasibility and potency of GAM plasmid gene delivery from a biodegradable, sustained-release polymer matrix to rat skin dermis. A high-pressure gas foaming process was developed to efficiently incorporate supercoiled DNA into three-dimensional porous matrices of poly(lactide-co-glycolide). Incorporated DNA was released over times ranging from days to a month in vitro. In vivo delivery of a plasmid encoding platelet-derived growth factor B led to a three- to fourfold enhancement of granulation tissue at the implantation site. This result was contrasted with direct injection of the platelet-derived growth factor B plasmid, which did not significantly enhance local tissue formation, a result that emphasizes the utility of the matrix carrier.

Finally, Berry et al. (submitted) investigated the feasibility and potency of GAM plasmid gene delivery to sites of cranial nerve injury. When a GAM was placed between the proximal and distal stumps of severed rat optic nerve, it was shown that DNA was taken up at severed nerve ends. Plasmid gene uptake was enhanced by linking recombinant fibroblast growth factor 2 to plasmid DNA. Following uptake, the sustained presence of DNA, RNA, and recombinant protein in the retina (where regenerating axons originate) was demonstrated. Moreover, GAMs containing neural growth and survival factors promoted significant retinal ganglion cell survival for more than 3 months after injury. Together, these studies demonstrate that (a) wound healing fibroblasts can be genetically manipulated in vivo, (b) that plasmid DNA can be delivered to fibroblasts in skin from a biodegradable tissue engineering matrix, and (c) that plasmid gene delivery can be targeted in nerve via growth factors such as fibroblast growth factor 2.

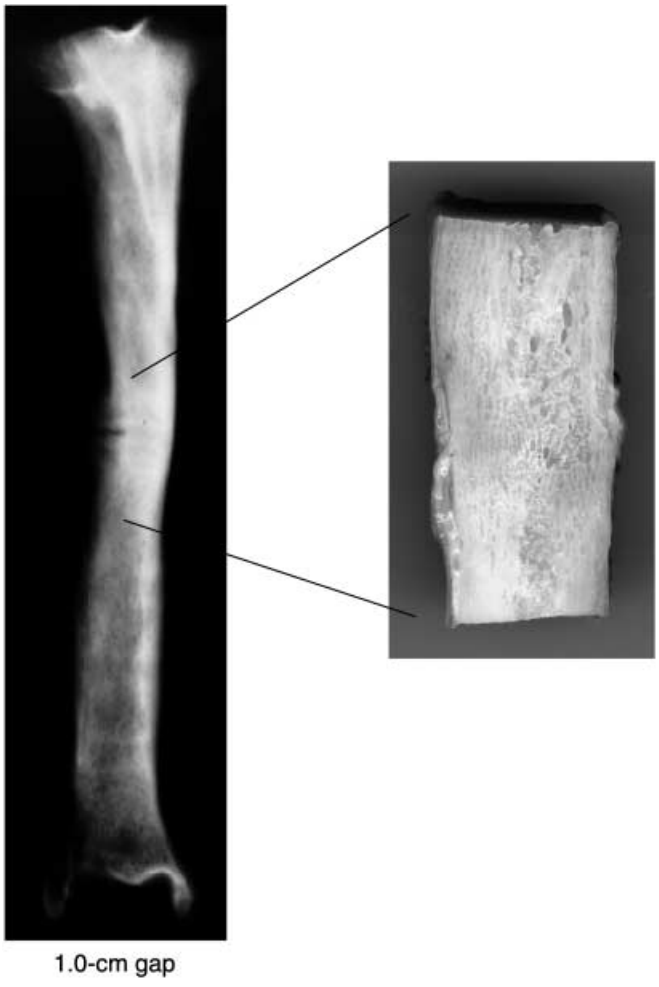

Fig. 3 The figure shows evidence of complete healing of canine bone defect. *Left* Radiograph shows a tibia with repaired segmental defect following GAM implantation (1.0-cm gap); *right* morphological view shows 1.0-cm defect filled in with new bone following plasmid gene transfer

Application of the GAM technology to human medicine

While gene therapy has now been applied to a variety of medical disorders [22], perhaps the most difficult obstacle limiting gene therapy has been the inability to match, in a safe and effective manner, gene and gene-delivery systems with the appropriate clinical indication in human subjects. The failure to achieve approvable efficacy is thought to be both a delivery and a safety issue [23]. As regards delivery, a major concern is the ability to deliver the therapeutic gene to a specific cell population in a safe and effective manner.

How should the GAM technology be applied to human medicine? There currently is no direct evidence to suggest that GAMs significantly accelerate wound healing. As demonstrated in canines [15], the major advantage may be that local plasmid gene delivery engenders a dose-dependent, reproducible, and safe tissue regeneration response. Therefore GAM is probably best considered as a method to achieve wound healing when the wound healing response is inadequate. ("Inadequate" would be defined empirically from medical practice.)

Given the experimental nature of the GAM technology, the chosen medical indication for "first use in man" should be significant in terms of morbidity, mortality, and societal impact [24]. Moreover, patients who participate in GAM clinical trials should not be denied the current standard of medical care, i.e., GAM delivery should not significantly alter the preferred treatment protocols for the medical clinical condition of interest. Finally, the GAM formulation of interest, as well as the recombinant protein product of the GAM plasmid gene, should be demonstrated to be both safe and effective in rigorous preclinical studies.

At the time of this writing, the GAM formulation used to treat the first human subjects will most likely be Mat-100, a single-application, plasmid gene therapy for bone fracture repair in elderly human subjects who suffer from osteoporosis. Mat-100 is a lyophile that consists of plasmid DNA in a biodegradable bovine type I collagen sponge. In the operating room the Mat-100 lyophile will be removed aseptically from a vial (i.e., with a forceps or similar tool), placed near the fracture site by the hand of the surgeon, and then molded to fit the bone defect. (Mat-100 will be administered in conjunction with the placement of a medical device designed to stabilize the fracture site.) After delivery Mat-100 lyophiles should remain localized at the site of implantation until biodegradation is complete.

Below I discuss the rationale for the initial Mat-100 therapeutic trial. As discussed below, one factor that greatly influenced the decision to focus on osteoporotic fracture repair was the plasmid DNA component of Mat-100, which codes for the hPTH 1-34 peptide. The anabolic effect of hPTH 1-34 has been known for more than 70 years, and hPTH 1-34 is perhaps the best studied and best understood human bone growth factor, especially in terms of its anabolic effects on the osteoporotic skeleton. (Most would agree that the next best studied bone growth factor is bone morphogenetic protein, which was identified as an activity in the 1960s, was cloned in 1988, and has been studied in humans for about a decade.) The remainder of this review focuses briefly on the public health significance of osteoporosis, the current unmet clinical need associated with fracture of the hip in elderly osteoporotic individuals, and the evidence that hPTH 1-34 is a safe and effective bone growth agent for the elderly skeleton.

Osteoporosis

Low bone density, or osteopenia, is the sine qua non of osteoporosis. Over time, osteopenic bone undergoes progressive, microarchitectural deterioration, which some investigators refer to as fatigue degradation. In turn, fatigue degradation leads to the significant clinical risk that minimal trauma – which nevertheless exceeds the breaking strength – will be associated with bone fractures. Osteoporotic fractures most commonly occur in the hip, spine, and wrist.

Osteoporosis is now recognized as a major public health problem, i.e., on a par with atherosclerotic cardiovascular disease, hypertension, cancer, and diabetes. Osteoporosis currently is responsible for 1.7 million fractures per year in the United States, which is a staggering number. (In comparison, 1.2 million new cancers are diagnosed in the United States each year.) Health care expenditures associated with all osteoporotic fractures for white aged over 45 years have been estimated from prevalence-based cost-of-illness methods and data from 1995 health care surveys in the United States [25]. Of the 432, 448 hospitalizations in 1995 with a primary diagnosis of osteoporotic fracture in persons over 45 years old, 57% were for hip fracture, 6.8% were for spine fracture, and 3.1% were for forearm fracture. Fractures at other sites represented the remainder of cases. In all, 78.9% of hospitalizations were for women. An estimate of 4.1 million days in-hospital was reported, with an average of 9.6 days per hospital stay. On average, when compared to others lacking the diagnosis, patients with a secondary diagnosis of osteoporotic fracture remained hospitalized 4.4 days longer. Hospitalizations with a secondary osteoporotic fracture diagnosis contributed an added 509,136 days in-hospital for the 1995 calendar year. Additionally, 179,221 nursing home stays associated with 44.6 million patient days were attributed to osteoporotic fractures in 1995. Hip fracture accounted for 76.9% of these stays and 72.5% of the attributed days. White women represented a majority (75.9%) of these nursing home residents. A total of 3.4 million emergency room visits and outpatient examinations (i.e., physician office and hospital clinic) were attributed to osteoporotic fractures in 1995. Diagnostic imaging and physical therapy services were provided during 55.2% and 5.6%, respectively, of the outpatient examinations. The total number of physical therapy sessions for the

year was 193,557. In addition, a total of 2.4 million prescription and nonprescription medications were prescribed for osteoporotic fracture patients. Nearly 22,000 ambulance encounters were attributed to osteoporotic fractures in 1995, and nearly 500,000 orthopedic and other medical supplies were provided as treatment. Finally, approx. 2.2 million home health care visits were made for patients with osteoporotic fractures during the year.

From these data the health care expenditure for osteoporotic fractures in 1995 was estimated to be US $13.76 billion [25]. Health care expenditures were greatest for patients aged 65–84 years (52.8%), followed by patients aged 85 years or older (34.8%), and then patients aged 45–64 years (12.4%). Expenditures estimated by type of service include $8.6 billion (62.4%) for hospitalization, $3.88 billion (28.2%) for nursing home care and $1.3 billion (9.4%) for outpatient services. The latter included care that was received in emergency rooms and physician offices. By type of service and age, inpatient expenditures attributed to osteoporotic fractures were greatest for 65- to 84-year-old patients. Nursing home expenditures were greatest for patients aged 85 years or younger. Outpatient expenditures were greatest for patients aged 45–64 years. These data are consistent with those from four other studies [26, 27, 28, 29], in which the total expenditure for osteoporotic fractures was estimated to be between $7.3 and $12.4 billion in 1995 dollars. The data also are consistent with high-quality data obtained from the third National Health and Nutrition Examination Survey [30]. This survey in the United States uniquely examined the correlations of bone densitometry data with age, gender, ethnicity, and geographic region in more than 6,000 adults. Because the standard to determine fracture threshold was not clear at the time, two independent approaches were taken. These efforts in particular defined for the first time the incidence of osteoporosis in American men and nonwhite women, i.e., a 16% prevalence of osteoporosis in the proximal femur of Hispanic women and 10% prevalence in African-American women. Therefore these data must be added to the 1995 burden of $13.76 billion before true costs are known.

Osteoporosis is now a cause for concern in less developed nations [31, 32, 33, 34]. The population explosion in these countries, combined with a decrease in infant mortality and an increase in longevity, should contribute to the worldwide incidence/burden of osteoporosis. For example, one projection is that the number of osteoporotic hip fractures worldwide will increase from 1.7 million in 1990 to 6.25 million in 2050 [32]. Presently about one-half of all hip fractures occur in the United States and Western Europe. By 2050 it is predicted that these regions will account for only one-quarter of the total, and the large majority of hip fractures will occur in Asia and Latin America. There is a concern that these trends, plus the high medical costs associated with hip fracture repair, will have a significant negative impact on healthcare delivery systems and the general economies of underdeveloped countries in these regions of the world unless effective forms of treatment are developed.

Osteoporotic hip fracture

A majority of the costs, morbidity, and mortality associated with osteoporotic fracture are attributable to fracture of the hip. Hip fracture accounts for more than 50% of all osteoporosis-related hospital admissions among women over 45 years old in the United States [25]. In the United Kingdom hip fracture patients occupy 20% of all orthopedic beds, at a direct cost in England and Wales of £160 million per year in 1988 [35]. In France 56,000 hip fractures annually cost approx. 3.5 billion francs [36]. In Australia 10,150 hip fractures in 1986 cost an estimated Australian $38 million [37]. These are formidable burdens on health care, but costs will rise based on what we know of the demographics of aging: life expectancy is increasing in every geographic region worldwide. In the United States the number of individuals over 65 years old is expected to rise from 32 to 69 million between 1990 and 2050 and the number of individuals over 85 years old will grow from 3 to 15 million. Currently the 323 million individuals worldwide who are over 65 years old will increase to an estimated 1,555 million by the year 2050 [32]. Growth in the elderly population will be greatest in Asia, Latin America, the Middle East, and Africa. The influence of the anticipated changes in population on the number and regional distribution of hip fractures will be dramatic. Hip fracture incidence rates increase exponentially with age, and the estimated number of hip fractures in the United States could double from 238,000 in 1986 to 512,000 in 2040 [38]. However, the elderly population in the United States has grown more rapidly than predicted, and if these trends continue, the number of elderly individuals in the year 2040 could be 22% higher than currently anticipated, and the resulting number of hip fractures in the United States could total 840,000 [39]. In 30 years' time the annual direct cost for hip fracture care in the United States could rise by 50% in constant dollars [38], or by more than twice if the population continues to age more rapidly than expected [39].

Operative management is the treatment of choice for the great majority of hip fractures (for the most authoritative text see [40]). Ideally, fracture fixation should allow for a return to normal (baseline) weight-bearing ambulation as tolerated because the elderly have difficulty with non-weight-bearing- and partial weight-bearing ambulation. For the great majority of patients, fracture fixation and fracture healing is achieved by placement of an orthopedic device made of titanium or stainless steel.

The morbidity associated with hip fracture is related in part to osteopenia at the fracture site [40, 41, 42, 43, 44, 45, 46]; osteopenia leads to a less stable and less functional hip in spite of current treatment regimes. This is because of the relative inability of screws and pins to gain adequate purchase of osteopenic bone stock and because of a decline in the fracture healing/bone regeneration capacity as a function of age. The most common orthopedic complications after intertrochanteric fracture fixation are varus displacement of the proximal frag-

ment, malrotation deformity, and nonunion. Osteonecrosis, disengagement of the screw from the barrel, and migration of the screw into the acetabulum are uncommon occurrences [40]. Varus displacement after initial fixation is associated with unstable fractures as a result of the lack of posteromedial support. With the sliding hip screw, varus displacement usually results in the screw cutting out through the anterosuperior portion of the femoral head. Other associated complications include breaking or bending of the implant, screw penetration into the joint, and disassociation of the plate from the shaft.

Reliable data are not available, in part because there is no accepted method of evaluating fracture healing in human subjects, but it is reasonable to believe that 5–20% of hip fracture patients worldwide suffer one or more of the complications described above. (As osteoporosis grows more important this number may actually increase, i.e., especially in less developed nations with relatively unsophisticated fracture treatment regimes.) When complications occur, management choices include a second attempt at open reduction and internal fixation, acceptance of the deformity and the decrement in ambulatory function, and hip arthroplasty [40]. These complications are serious, contributing directly to lost productive quality of life. It is significant that approx. 50% of the elderly who were ambulatory before hip fracture are unable to walk without assistance after hip fracture, and that approximately 25% of the elderly who fracture the hip require long-term domiciliary care. In an unpublished study of 682 hip fracture patients, Magaziner and colleagues found that the gait and balance of hip fracture patients, measured in the 24 months following standard orthopedic treatment, was about 50% of that expected for age-matched controls. Finally, hip fracture patients show a 5–20% increase in mortality from expected survival during the first 6–12 months after fracture.

Is hPTH 1-34 a rational drug for hip fracture repair?

Perhaps the most direct answer to this question comes from the canine study [15] in which local plasmid gene transfer via Mat-100 prototypes was used to fill surgical defects in tibiae of intact, skeletally mature beagles that mimic acute bone fracture in humans. New bone filling these defects persisted well beyond 2 years postsurgery without radiographic or clinical evidence of bone loss. The results of this study suggest that once the optimal DNA dose range is identified, hPTH 1-34 is a reproducibly effective and safe anabolic agent for fracture repair. However, given the desire to treat osteoporotic hip fracture with Mat-100, an important limitation of this work is that the beagles were young, metabolically intact adults. Therefore an important unknown is whether hPTH 1-34 will be an effective bone growth agent in the osteoporotic skeleton. Fortunately, an extensive and relevant database helps answer this question.

A dose-response for hPTH 1-34 peptide and new bone formation in the osteopenic skeleton has been clearly established in the literature [47]. Osteopenic rats have been treated with hPTH 1-34 at doses ranging from 0.5 to 1000.0 μg/kg body weight. The most common effective dose appears to be 80.0 μg/kg, administered by subcutaneous administration at least three times per week, but lower doses (30.0–60.0 μg/kg) have also produced consistently positive results. Shen et al. [48, 49] used a low dose of hPTH 1-34 (2.5-μg/kg) in osteopenic rats to be consistent with the equivalent dose used in clinical trials in osteoporotic human subjects. This low dose stimulated bone formation somewhat and partially restored lost bone (in osteopenic rats). Protocols that employ high doses (400.0–1000.0 μg/kg) have strong anabolic effects, but some of the new bone is woven rather than lamellar and marrow fibrosis (a potentially undesirable side effect). The common effective dose has been shown to increase the load to failure of bone specimens from osteopenic rats beyond that of sham-operated intact controls. More recently studies in other animal species, including osteopenic adult female cynomologous monkeys, showed that hPTH 1-34 induced a rapid increase in bone mass at the spine and hip that was associated with increases in biomechanical strength [50]. hPTH 1-34 was administered in two doses (1 and 5 μg/kg) as a daily subcutaneous injection for 12 months. A dose response was observed, with no evidence of a safety concern. Finally, data from current and ongoing human clinical studies [51, 52] show unequivocally that hPTH 1-34 is rapidly, significantly, and reproducibly anabolic for both cortical and trabecular bone. Moreover, significantly higher doses of hPTH 1-34 peptide than will be expressed after plasmid gene delivery have been administered to the bloodstream on a daily basis for years and shown to be both safe and effective.

It is well established that PTH anabolic effects are greatest if the peptide is administered intermittently. Although continuous administration increases bone formation in intact rats [53, 54], resorption is increased to roughly the same extent as formation, so that bone amount/density overall is unchanged (or actually decreased). In contrast, intermittent treatment (i.e., subcutaneous injection of hPTH 1-34 peptides) markedly stimulates net new bone formation, amount, and density [47]. While the data are lacking, it seems unlikely that hPTH 1-34 expression from GAM is pulsatile, and yet bone formation is robust in our rat, canine, ovine, and equine preclinical fracture models (e.g., [15]). Therefore the manner of hPTH 1-34 administration from GAM to a fracture site, as it relates to the efficiency of the bone regeneration response, remains an important area of future research.

Conclusion

This review was written with two objectives in mind. The first was to describe the GAM technology and its

potential rational use in tissue regeneration and tissue engineering. Development of the GAM technology has been an interdisciplinary exercise, involving the disciplines of bioengineering, drug delivery, and gene therapy. GAM was the first gene therapy designed specifically for tissue engineering applications, and the mechanism of action of plasmid gene transfer is closely tied to the normal sequence of events associated with wound healing. The general outline of this sequence is faithfully reproduced in a wide variety of wounded tissues and organs. Therefore the hypothesis pursued in the first GAM studies was that the technology could be broadly applied (i.e., if it worked in bone, it may also work in skin and nerve, etc.). This hypothesis has essentially been proven based on animal model studies of acute tissue injury in bone, skin, nerve, tendon, ligament, and muscle [11].

The second goal of the review was to describe the most likely "first use" of the technology in man. Although not universally appreciated, I believe that orthopedic surgeons will be interested in osteoporosis because these fractures are commonplace (i.e., most of their patients will be affected sooner or later) and because of the devastating impact of osteoporotic fractures on morbidity. It perhaps is not surprising therefore that the current decade (2000–2010) has been declared the Bone and Joint Decade by the World Health Organization [55], in part because of the significance of osteoporosis. (After all, an estimated complication rate of 5–20% for osteoporotic hip fracture equals a global unmet clinical need of 85,000–340,000 cases today and an expected 315,00–1,260,000 cases in 2040.)

The hypothesis of the clinical trial that represents the "first use" of the GAM technology in man is that the morbidity associated with hip fracture can be significantly reduced by therapy that aims to promote bone regeneration at the site of fracture fixation. The suggestion is that new bone growth at the fracture site will lead to enhanced stability, thereby allowing for more aggressive rehabilitation, greater confidence, and increased functionality. Obviously, GAM is at an early stage of development, and much remains to be learned about its safety and efficacy. Additionally, a successful commercialization strategy has yet to be developed. In this regard, many significant problems, in the United States in particular, are associated with reimbursement by the Federal government for medical care of the elderly. Nevertheless, the market opportunity for innovative therapies that significantly increase the standard of care of elderly patients with osteoporotic fractures is enormous. Moreover, if successful in bone, it may possible to use localized gene therapy for tissue engineering to regenerate other tissues as well.

Acknowledgements I thank Paul De Stefano, Laura Coruzzi, Peter Hoyle, and Ze'ev Shaked for support. I also thank Janet Hock for a critical review of the manuscript. Studies by the author were supported by grants from NIH and by a Sponsored Research Agreement from Matrigen, Inc. to the University of Michigan.

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