Bone xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Bone

journal homepage: www.elsevier.com/locate/bone

Review

Stem cells and bone: A historical perspective

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ARTICLE INFO

Article history:
Received 18 May 2014
Revised 19 August 2014
Accepted 20 August 2014
Available online xxxx

Edited by: Frank P. Luyten

Keywords:
Bone
Stem cells
Skeletal stem cells
Mesenchymal stem cells
Hematopoietic niche

ABSTRACT

Bone physiology and stem cells were tightly intertwined with one another, both conceptually and experimentally, long before the current explosion of interest in stem cells and so-called regenerative medicine. Bone is home to the two best known and best characterized systems of postnatal stem cells, and it is the only organ in which two stem cells and their dependent lineages coordinate the overall adaptive responses of two major physiological systems. All along, the nature and the evolutionary significance of the interplay of bone and hematopoiesis have remained a major scientific challenge, but also allowed for some of the most spectacular developments in cell biology-based medicine, such as hematopoietic stem cell transplantation. This question recurs in novel forms at multiple turning points over time: today, it finds in the biology of the "niche" its popular phrasing. Entirely new avenues of investigation emerge as a new view of bone in physiology and medicine is progressively established. Looking at bone and stem cells in a historical perspective provides a unique case study to highlight the general evolution of science in biomedicine since the end of World War II to the present day. A paradigm shift in science and in its relation to society and policies occurred in the second half of the XXth century, with major implications thereof for health, industry, drug development, market and society. Current interest in stem cells in bone as in other fields is intertwined with that shift. New opportunities andalso new challenges arise. **This article is part of a Special Issue entitled "Stem cells and bone".**

Contents

Introduction

Bone morphogenetic proteins, hematopoietic "niche," and "mesenchymal" stem cells represent three totemic achievements in bone biology during the last century, three of the most research-intensive areas of the last three decades, and three of the most "translation"-intensive research areas of the present day. The three fields emerged from an unusual concentration in space and time of a handful of seminal experimental observations. In just a few years, we learned that heterotopic transplantation of transitional epithelium into skeletal muscle induces heterotopic bone formation [1]; that heterotopic transplants of bone marrow also do so [2,3], but that the two phenomena are radically distinct from one another: the former is dependent on the release of a soluble factor, while the latter is not. Identification of BMPs [4–6,7] and perisinusoidal reticular cells as the specific factor and cell type

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http://dx.doi.org/10.1016/j.bone.2014.08.011
8756-3282/

generating bone in heterotopic transplants of transitional epithelium and bone marrow, respectively, represents the ending point of two long and diverging journeys that originated from those seminal experiments. Likewise, the definition of the bone marrow microenvironment as the host of signals provided by stromal cells and required for hematopoiesis, and the pursuit of a "niche" for hematopoietic stem cells proper represent the developments over time of a third seminal observation; that is, that grafting of bone marrow in closed systems (diffusion chambers) would generate bone but bar the development of hematopoiesis, whereas transplantation in open systems would allow for both bone formation and development of marrow [2].

That all of these fundamental observations, which not only withstood the test of time, but also represented the seed for the subsequent flourishing of major fields of investigation, arose from the practice of heterotopic transplantation cannot escape notice. Considering the tremendous impact of establishing quail–chick chimeras (a kind of heterotopic transplantation in embryos) [8,9]in developmental biology and how much it contributed to further developments in lineage tracing, one is tempted by foolishly wondering what magic is inherent in putting tissues and cells where they do not belong (ectopic transplantation), and why is this practice so instructive. Perhaps all these simply highlightthe fundamental link between space (and time) and development (lineage, commitment, differentiation), a notion we owe, ultimately, to Alan Turing (the father, among many other things, of the diffusion–reaction model which established the chemical basis of morphogenesis [10]), and before him, to D'Arcy Thompson (a classicist and a morphologist renowned for his attention to the physical and mathematical laws underpinning morphogenesis) [11]. Heterotopic transplantation is instructive because it breaks the spatial and temporal constraints (the physics, one could naively argue) that drive development, and therefore reveals them in the most empirical way possible.

The fallout: post-World War II era

That these fundamental observations clustered in a specific stretch of time, on the other hand, is also intriguing. In the same, specific time interval, another major change in scientific trends arose. The idea of a hematopoietic stem cell, a common multipotent progenitor for all blood cells, had been formulated long before (reviewed in [12]), but had remained dormant without attracting interest and above all, experimental effort. The idea exited the realm of theoretical postulates in 1961, with the seminal work of Till et al.[13,14], admittedly the first experimental evidence for a common multipotent progenitor of blood cells. In essence, the fundamental discoveries of a dual system of stem cells in bone were not only almost synchronous, but also arose from efforts across the iron curtain that fell at the end of WWII, and are the direct result of the way WWII ended. It was the attempt to develop strategies for radioprotection that gave a new impetus to the science behind what was to become stem cell biology. Not casually, the front page of the famous New England Journal of Medicine paper by E. Donnall Thomas reporting in 1957 [15] the first attempt of bone marrow transplantation in humans both recounts the lethal effects of nuclear warfare, and acknowledges the support of the Atomic Energy Commission of the USA. Much more in bone science and science at large emanate from the same cradle: the biology of bone matrix [16,17] and the role of parathyroid glands [18], for example, and key techniques such as microradiography and autoradiography [16,17,19–21], to name a few.

At about the same time that something "osteogenic" was being discovered in bone marrow by Tavassoli and Crosby [3], and by Friedenstein and coworkers [2], it was exactly autoradiography that made it possible to trace the kinetics of bone cells in vivo, in a series of seminal studies by Owen and Macpherson [22–25]. This is how we learned about precursor cells of osteoblasts in the inner layer of the periosteum, about the origin of osteocytes from osteoblasts, and about the kinetics thereof. Not casually, the two independent lines of thinking about the origin and precursors of bone cells were to merge soon thereafter in the work of Owen, just like her background in physics and attention to biology had merged in her early work as a reflection of the post-war climate and strategic priorities. Even the work of Friedenstein and that of Owen united at one point [26], which was crucial to disseminate the significance of Friedenstein's work in the West (Figs. 1 and 2). That unification was also crucial to formulate the concept not only of a stem cell for bone, but also for different tissues together comprising the skeleton being connected to one another at the level of a common ancestor, rather than as separate entities as thought previously. For the first time, chondrocytes, osteoblasts and bone marrow adipocytes were brought together into a unified system. The "stromal system" comprising them all was conceived on the blueprint of the hematopoietic system, marking a major conceptual novelty in skeletal research [26,27].

The road to stem cells

Earliest experiments provided evidence for an inherent osteogenic potential of cells in bone marrow, and for its non-humoral nature. Subsequent steps involved the use of cell culture as a way to separate, at a time when no cell sorting tools were at hand, hematopoietic cells proper from non-hematopoietic (stromal cells), which in contrast to the former can adhere to a plastic substrate. Transplanting cultured stromal cells to the effect of generating heterotopic bone proved that it was the stromal fraction to be endowed with osteogenic potential. Using the same experimental approach, the same potential was later ascribed to the clonogenic fraction of stromal cells (i.e., to cells capable of density-insensitive clonal growth and therefore seen as progenitors), and to a subset of individual clonogenic cells [28–30]. The coexistence of multiple tissues within heterotopic "ossicles" generated by single clones proved the existence, first in rodents and much later in humans [31], of multipotent stromal progenitors, based on which the idea of an osteogenic stem cell was formulated as a working hypothesis [26,27,32]. Proving the existence of a bona fide stem cell also required proving the ability of the multipotent progenitor to self-renew, but this key question remained unaddressed for many years. Addressing this question required the identification of an anatomical in vivo counterpart of the multipotent clonogenic progenitor, and proof of its regeneration in heterotopic transplants. This only came with the demonstration that: a) the clonogenic fraction of bone marrow stromal cells in humans coincides with perisinusoidal reticular cells; which b) could be pinpointed using immunocytochemical markers both in the intact bone marrow and in the heterotopic graft; and c) could be secondarily isolated from the grafts, expanded and serially transplanted. First provided in humans [33], this type of evidence was later provided in the mouse [34]. Completion of this pursuit over 40 years leaves us with the notions that indeed, clonogenic, multipotent and self-renewing progenitors for

Fig. 1. Alexander Friedenstein.

Please cite this article as: Bianco P, Stem cells and bone: A historical perspective, Bone (2014), http://dx.doi.org/10.1016/j.bone.2014.08.011

Fig. 2. Maureen Owen.

skeletal tissues reside at the abluminal surface of bone marrow sinusoids as "adventitial reticular cells," [33] which are the in situ counterpart of explantable clonogenic stromal cells. These cells play a key role in establishing the hematopoietic microenvironment, and, possibly, the "niche" for hematopoietic stem cells.

Which cells are which?

Taken together, the results of this long experimental history provides much clarity as to the identity not only of the long sought-after skeletal stem cells, but also of all other "cells" that one handles as natural or technological objects revolving like planets in the "stromal system." "Osteoblasts," of course, remain the differentiated cells that deposit and mineralize the bone matrix; "stem cells" are the self-renewing and multipotent progenitors; "stromal progenitors" are the clonogenic stromal cells; "stromal cells" are all the non-endothelial, non-hematopoietic cells other than mature osteoblasts or smooth muscle cells that exist in situ in the intertrabecular space in bone, that one can establish in culture as adherent cells; "stromal cell cultures" are cultures of all stromal cells, regardless of whether they are established from total bone marrow cell suspensions, multiple colonies generated by stromal progenitors, single clones, or phenotype-purified ("prospectively isolated") stromal cells [35] (Table 1).

Stem cells for bone

No doubt, recognizing that bone is a living tissue rather than simply a hard object, was a major advance in bone science, giving birth to the fundamental idea that bone has a metabolism and that cell dynamics make it possible. Recognizing the duality of bone construction and deconstruction, of cells behind each action, and later of their dual developmental origin gave bone a physiological dimension that exceeded a merely mechanical function. This brought consideration of bone physiology into internal medicine. Bone formation and resorption and the dynamics thereof became the fundamental tenets of bone research, focusing the attention on bone remodeling as essentially the sole cell-based dynamics therein, or the only relevant one. Measurement of

Table 1
Which cells are which? A mini-glossary.

Osteoblasts	Cells that directly deposit a mineralizing bone matrix on a nascent bone surface
Bone marrow stromal cells	In situ, cells of non-hematopoietic, non-endothelial nature that provide the stromal scaffold and the host of cues and signals supporting hematopoiesis, in the extravascular space of bone marrow In vitro, all cultures generated by explanted stromal cells, including those generated by total cell suspensions, by progenitors selected by plastic adherence at clonal density, or by phenotype-purified explanted cells
Clonogenic stromal cells	The subset of stromal cells capable of initiating clonal density-insensitive growth. A progenitor cell, not necessarily a stem cell. Some clonogenic stromal cells are progenitors; some are multipotent progenitors; some are multipotent and self-renewing stem cells.
Skeletal stem cell	The multipotent and self-renewing stromal progenitor, which can be shown in vivo to give rise to multiple skeletal tissues (bone, cartilage, marrow adipocytes); resides over bone marrow sinusoids; can re-establish, in vivo, a compartment of clonogenic multipotent progenitors residing over sinusoids, with identical phenotype; can be secondarily passaged and/or serially transplanted.
Bone marrow stromal, osteogenic stem cells	Original denominations by Friedenstein and Owen for the putative multipotent stem cells underpinning the property of stromal cell clones to generate multiple tissues in vivo, such as bone and cartilage, hematopoiesis-supportive stroma and marrow adipocytes
Colony-forming unit-fibroblastic, CFU-F	A single clonogenic stromal cell
Mesenchymal stem cell	Originally, the same entity as the putative "osteogenic" or "stromal" stem cell in the bone marrow, with additional putative properties such as those of progenitors of skeletal muscle, tendon, or fat. Subsequently, cultured cells defined by in vitro criteria only, and isolated from any source

those dynamics (histomorphometry) [36] came to center stage in bone research. For the same reason, contemporary cell biology in bone arose from efforts to establish osteoblasts [37,38] and osteoclasts in culture [39], reflecting directly the general focus on differentiated cells and their functions as the physiological basis of bone remodeling. Bone mass, viewed as the result of the equilibrium between formation and resorption of bone, became the single most important variable in bone anatomy, while osteoporosis became the single most important bone disease dominating "bone medicine." The pharma industry, the size of a market coinciding in principle with the adult female population, and political and social interest in a disease largely prevalent in women all contributed to shape the biological view of bone during the 1980s and 1990s. Even so, the idea that skeletal progenitors matter gained impact and momentum, slowly but progressively. For example, cultures of bone marrow stromal cells gradually replaced cultures of "osteoblasts" in bone research, even in osteoporosis research, until they became the dominant tool for cell biology of human bone at least.

Turnover oddity

The concept of postnatal stem cells, at the time when a stem cell was envisioned for the skeleton, was inextricably linked to the self-renewal of high turnover tissues such as blood and epithelial tissues. The existence of bone turnover, and the ability of bone to regenerate after a fracture, were both invoked in support of the new concept. However, compared to blood and epithelial tissues, bone is a slow turnover tissue. While the epidermis turns over in its entirety once a month, the skeleton is completely replaced by a new one (or, an equivalent mass of tissue) 3–5 times in a lifetime (between skeletal maturity and death). One would argue that a stem cell could be dispensable for coping with this

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specific physiological need. Stated in a less teleological way, one would wonder why a system of stem and progenitor cells would be evolutionarily selected and conserved in the skeleton. Similar considerations, many years later, apply to many other systems seen today as dependent on some kind of stem cell. For example, we consider that a neural stem cell exists in specific regions of the brain, even if postnatal neurogenesis is very limited in rodents, and its very existence is still open to question in humans. Most importantly, we have extended significantly the use of the term "stem cell" beyond its original definition, which was tailored on postnatal self-renewing tissues. Attempts to define a set of functions as defining all kinds of cells we call stem cells have met a limit. Embryonic pluripotent stem cells (ES cells) and postnatal stem cells display majorly different biological properties. No postnatal (stem) cell is pluripotent, unless modified into an Induced Pluripotent Stem Cell. As applied to cultured ES cells, furthermore, the term self-renewal has a different meaning compared to the one it has in postnatal stem cells. Unlike postnatal stem cells, ES cells do not self-renew in vivo for the lifespan of the organism. Pluripotency can however be maintained in ES cells as these are cultured as continuous lines in vitro, under specific conditions. The extended use of the term "stem cell" (and of the terminology describing stem cell properties) for vastly different biological system calls, in fact, for a more precise appreciation of the physiological function that is encrypted in each kind of stem cell, and evolutionarily conserved. For embryonic pluripotency, diapause (the ability of some species to arrest embryo development and to resume it depending on environmental and nutritional conditions) can be tentatively conceived as the function conserved across a number of species, but not in primates [40]. For other systems, specific conserved functions remain to be identified, and each is linked to gross properties of the relevant "stem" cell system (growth kinetics, differentiation potential), and to the underpinning regulatory circuits. Identifying the properties and circuits that define the stem cells in bone rests not on the analogy, but on the divergence of the system from the hematopoietic system. For example, while the lineages emanating from the hematopoietic system (such as erythropoiesis, granulopoiesis) can be seen as existing in parallel, and being generated constantly at any time point, their "homologous" lineages in the stromal system (such as osteogenic, adipogenic) are not at all generated synchronously; e.g., chondrogenesis is predominantly a prenatal event in skeletogenesis, while adipogenesis is entirely postnatal [41]. Furthermore, a wealth of evidence, albeit circumstantial in large part, highlights the ability of individual cell types regarded as differentiated to modulate into different phenotypes. For example, chondrocytes can revert to fibroblasts [42,43] or osteoblast-like cells in vitro and in vivo [44,45], or even to bone marrow stromal cells in vivo [46]; bone marrow stromal cells can convert into adipocytes in vivo [47]. This "plasticity" of the stromal system (not to be confused with the once claimed, and now luckily dispelled, "trans-differentiation" ability of any cell to generate any cell, "turning blood into brain" [48], "brain into blood" [49], "blood into muscle" [50], "muscle into blood" [51], and water into wine [52]) remains to be understood mechanistically, but may be seen as one defining feature of the system and of its unique nature. Nonetheless, the differentiation potential of skeletal stem cells is strictly limited to phenotypes that belong to the skeleton: cartilage, bone, fat, fibroblasts and the bone marrow stroma itself are the only progenies of the marrow stromal stem cells. Skeletal stem cells, like all other kinds of postnatal stem cells, are committed and system-specific, and are not pluripotent. Finally, all cell types in the stromal system exist within an extracellular matrix. This is another noted peculiarity of the stromal system compared to other stem cell-dependent tissues such as blood or epithelial tissues. As the extracellular matrix embodies differentiation cues, maintenance of an individual phenotype within the stromal system is partly regulated "in trans"; constant remodeling of the extracellular matrix makes the "in trans" determination of phenotype inherently unstable. This instability may have been conserved as a specific adaptive function, other than constant and fast cell replacement such as in blood or epithelial tissues. These adaptive responses include the integrated remodeling of hard tissues with that of soft and fluid tissues. Following the disruption of soft tissue remodeling by ablation of the pivotal protease for collagen degradation, MT1-MMP, vicarious remodeling of bone disrupts skeletal integrity [53]. The adaptive co-regulation of skeletal and hematopoietic physiology involves remodeling of the bone marrow (e.g., timed generation of yellow (adipose) marrow during postnatal growth and aging, and local vascular remodeling) [54]. In a way, one of the notions that come from the existence of skeletal stem cells and the stromal system is that remodeling of bone is part of a much broader adaptive response, which involves the coordinated remodeling of other connective tissues. Just as much as an unbalanced remodeling of bone alone results in a disease we call osteoporosis, disruption of soft tissue remodeling results in a disease of bone and joints that we call Winchester's syndrome, for example [55].

Bone and the HSC niche

The notion that bone would include specific, saturatable sites for homing of hematopoietic stem cells and for their retention in a "stem cell" state was first proposed by Schofield [56]. The seminal work of Dexter, Allen and co-workers [57] highlighted the role of bone marrow stroma in the maintenance of hematopoiesis and hematopoietic stem cells in a defined in vitro model, further highlighting a specific function of bone of major physiological significance. Revival of the interest in this function over the last 10 years came from two seminal studies in 2003 [58,59] showing that genetic manipulation of bone cells in the mouse can result in an increase of assayable hematopoietic stem cells. While this effect was initially attributed to osteoblasts proper, effects of the structural changes induced by transgenesis and of other cell types in the osteoblastic lineage could not be strictly ruled out. Subsequent studies showed that establishment of hematopoiesis in heterotopic transplants of human skeletal progenitors is dependent on the sequential establishment of bone and a sinusoidal network, and on the self-renewal of a subset of transplanted cells into perisinusoidal stromal cells. However, establishment of hematopoiesis is not directly coupled to establishment of mature osteoblasts and bone per se in the grafts [33]. In these systems, phenotypic long-term hematopoietic stem cells of the host colonize the graft in significant numbers, along with a complete array of assayable hematopoietic progenitors and lineages [46]. Similar studies in the mouse also pointed to a specific role of skeletal (mesenchymal) stem cells as "niche" cells [34], further promoting the search for a niche cell coinciding with a perivascular stromal progenitor in the mouse, and identifiable by a specific marker (e.g., nestin or leptin receptor) [60–62]. That bone and hematopoiesis are two interacting systems rather than just two strange bedfellows can be seen as a classical notion, perhaps underappreciated. The new data generated in the last ten years, however, directly point to a dual system of stem cells interacting with each other, a scenario that finds only rare matches in Drosophila [63], but otherwise quite unique in vertebrate systems. However, Schofield's concept of the niche as a fixed saturatable micro-anatomical site, while still pursued in the form of individual niche cells, expressing individual genes and proteins, was based on assumptions that reflect a specific set of data obtained in a specific experimental layout, and also the mindset of hematology at large; that is, on data based on transplantation of hematopoietic progenitors into a "bone" assumed to be a fixed entity. In a "bonehead" mindset, bone remodels, and so does the marrow stroma, along with the vascularity common to both bone and marrow. Furthermore, the transplantation of stroma reverses the logic of hematopoietic progenitor transplantation; the latter recapitulates hematopoietic ontogeny against a fixed microenvironment; the former recapitulates the ontogeny of the microenvironment against a fixed, steady state hematopoiesis. It is blood-borne hematopoietic progenitors that populate heterotopic bone organoids, and they do so while the organoid develops. Therefore, heterotopic transplants represent the only model available in which human bone marrow can be dynamically investigated as it develops. The niche might coincide with a

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developmental process more than with a definable microentity; past the developmental stage, it would remain as being dispersed across the skeleton, and subject to constant remodeling and adaptation events involving multiple cell types within, precisely, the stromal system. Implications of the niche concept for disease, however, are huge. They involve hematopoietic and non-hematopoietic cancer, their development and local promotion; myeloproliferative and myelodysplastic syndromes; and of course, the kinetics of homing and engraftment of hematopoietic progenitors as used in clinical protocols [64].

Stem cells and bone medicine

Understandably, the first applicative use that was envisioned as a result of the notion of stem cells for bone and other skeletal tissues was their use for engineering bone and other skeletal tissues [65–68]. This remains a highly viable avenue, rooted into a simple and solid concept with more than a reasonable amount of solid biology behind it. The ability of bone marrow stromal cells to generate histology-proven bone in vivo by local transplantation has been repeatedly proven by a number of laboratories around the world (reviewed in [69]), using a number of variations of the same fundamental approach. Indeed, the idea of using these grafts orthotopically for reconstructing skeletal segmental defects [67] represents a direct extension of the very assay used for proof-of-principle. Issues at hand include systems for efficient scale-up that allows for retention of the fundamental, desired property (osteogenic capacity), or the design of the optimal construct combining cells and biomaterials. Much of the initial delay in the latter area came from the adoption of paradigms that were borrowed from the previous era of (cell-free) bone tissue engineering, such as the need to design "porous" scaffolds to allow for vascular ingrowth. Organization of an efficient vascularity within the graft-generated tissues is crucial, but may be thought of in a more dynamic way in which space captured by the scaffold may not be essential. In view of the perivascular location of skeletal progenitors in experimental heterotopic grafts [33], it also follows that the development of a proper vascularity must include the establishment of a reservoir of skeletal progenitors in the graft [70]. Recent developments have generated a variety of approaches for the choice of material and the design of scaffolds, and a noted promising development rests with the potential use of constructs in which the scaffold coincides with a "natural" extracellular matrix made by the same osteoprogenitor cells [46,71,72], which may recapitulate, to some extent, processes operating in natural bone development, including the establishment of a perivascular compartment of functional progenitors.

This first-generation use of stem cells in surgery was followed by the attempt to target the skeleton systemically through intravenous infusion, in order to treat systemic (genetic) skeletal diseases [73]. This approach was not as biologically grounded as the surgical approach, given the inability of systemically infused skeletal stem cells to home routinely and efficiently to the skeleton [74]. Strategies to improve homing of skeletal stem cells are being pursued [75,76], as covered elsewhere in this issue. Of note, other hurdles would still stand in the way, even if the homing issue were resolved; that is, to reconcile the strategy of cell replacement with the slow turnover time of the skeleton. Regeneration of blood and epithelial tissues rests directly on their rapid turnover, which translates into rapid regeneration. In bone, turnover is slow, and regeneration would have to recapitulate development and post-natal growth of skeletal segment, but in a highly accelerated way.

Beyond the use of cells as therapeutic tools or vehicles, skeletal stem cells provide a novel angle on disease mechanisms, which might be targeted, in the end, by a pharmacological approach. More in general, the role that rare diseases have come to play in medicine cannot escape attention. Since the signing of the Orphan Drug Act signed by President Reagan in 1983, rare diseases have become a profitable pathway for pharma industry. In the same way as several drugs developed as "orphan" later came to represent innovation of much broader impact and with much broader market, rare diseases encrypt fundamental developmental mechanisms, targeting of which has often broad implications. Advances in understanding bone development have been spectacular over the past 30 years; capitalizing on these developments, and focusing on the cell biology of stem cells and the stromal system in bone predicts further advances in all those instances in which disease mechanisms rest on disruption of adaptive physiology of bone as an organ.

Bone and "mesenchymal" stem cells

The biological entity defined by the work of Friedenstein and Owen, and others, i.e. a putative stem cell for skeletal tissues found the bone marrow stroma, was renamed "Mesenchymal stem cell" in 1991 [77]. At about the same time, the first company was created to develop "mesenchymal stem cells" as a commercial product. The overlap of the "mesenchymal stem cells" in bone marrow with the biological object previously called "osteogenic" or "stromal" stem cell is obvious from the key papers that introduced "MSCs" [77,78]. It is also crystallized in the key criteria later issued for defining "MSCs" and widely accepted: i.e., their ability to generate bone, cartilage and adipocytes [79], the histological components of the bone–bone marrow organ that represent the progeny of skeletal stem cells as originally conceived. The introduction of the term "mesenchymal stem cells" coincided however with the introduction of a different biological concept. In the new concept, the putative "MSC" would represent a progenitor for both skeletal and extraskeletal derivatives of mesoderm, all viewed as part of "mesenchyme", all generated through a putative "mesengenic process" in development [77,80]. Mesenchymal stem cells would be entirely defined by in vitro properties and phenotype, gauged through non-stringent criteria and artificial in vitro assays (prone to artifacts and misinterpretation) [79]. In the mainstream inaugurated by the new views, others conceived the bone marrow stromal progenitor cells as stem cells for non-hematopoietic tissues [81] (quite a broad range of tissues of divergent lineage and functions), including derivatives of germ layers other than mesoderm such as neurons or liver cells, making "MSCs" (or subsets thereof) a postnatal version of pluripotent cells [82,83]. These initially appealing concepts, unlike the concept of a skeletal stem cell, have not withstood time and experimental scrutiny and are no longer widely entertained. Nonetheless, they did have a lasting impact. Before the introduction of technologies for reprogramming somatic cells into genuine pluripotency, a number of attempts to regenerate non-skeletal tissues with "MSCs" were made in preclinical models and clinical trials. The hope to develop "novel therapies" for major diseases was the *leit-motif* of such attempts, which were based on an assumed (and yet never truly proven) ability of MSCs to generate non-skeletal cell types. Many of these hopes, in turn, failed to withstand serious scrutiny (see for example, the recent DAMASCENE metaanalysis on the use of bone marrow cells for ischemic heart disease [84]). Granting the status of "innovation from discovery" to what was merely a seductive but unproven hypothesis, however, contributed to promote with the public the unauthorized use of unproven cell therapies aiming at commercial exploitation of the severely ill — even very recently, even in affluent countries [85].

Complementary to the hypothesis that "MSCs" potential would not be restricted to skeletal tissues was the idea that MSCs could be found in non-skeletal tissues. This idea became prevalent about a decade ago as a result of the looking at multiple tissues using non-adequate biological criteria for identifying the stem cells being sought [79,86]. Following the identification of bone marrow skeletal stem cells (i.e., the archetypal "MSCs") as perivascular cells [33], the same experimental approach and the same conceptual implications were extrapolated to claim that perivascular cells ("pericytes") are the in situ counterpart of "MSCs" in all tissues [87,88]. Perivascular progenitors do exist in multiple tissues, including fat and muscle, both in humans and in mice. They do not represent "MSCs" or skeletal stem cells, however, but a diversified system of tissue-specific progenitors (reviewed in [35,69]). The applicative implications of either view are obvious: use of stem cells for bone

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regeneration, for example, is highly dependent on the genuine, inherent osteogenic capacity of the chosen cell population, which implies choosing the appropriate tissue source (bone marrow or periosteum, but not fat or muscle or umbilical cord). Downstream of their unwarranted equation with "all pericytes", more recent versions of the "MSC" concept capitalize on properties that pericytes may exert in physiology, but are not per se the functions of stem cells. Promotion or quenching of inflammation, wound healing, control of tissue trophism via regulation of blood flow, for example, can be seen as local functions of pericytes [89], but not of stem cells. These functions resonate in the "trophic, anti-inflammatory, immune modulatory" properties that are invoked to underpin the empirical use of infusions of skeletal (or connective tissue) cells in a broad range of severe non-skeletal diseases unrelated to one another[80,90], for which MSCs provide no chances of cure (reviewed in [35]). Such use of cell infusions outside of a precise paradigm for tissue regeneration, and in the lack of a rationale, has antecedents noted in the history of medicine [91,92], but no record of positive outcome or achievement. Some refer to the legacy of those century-old experiences, still reproduced for commercial purposes today, as "dark cell therapy", as opposed to mainstream tissue regeneration attempts.

Into the new history

It is impossible to grasp the origin and the general significance of these conspicuous trends in the science of bone stem cells without placing these trends into their context. Conversely, the evolution of the science of stem cells in bone provides perhaps the most effective example of the impact of societal trends on present-day science. The post-WWII paradigm of R&D in biomedicine, as outlined in the famous document by Vannevar Bush, "Science, the Endless Frontier" [93] had a pivotal role in creating the contemporary biomedical science that flourished in the West after WWII. This paradigm is currently replaced by the "translational" paradigm. It is indeed a historical change [94,95]. The change begins in the 1980s and it is intertwined with profound changes in Western economies, in industrial strategies, in private and public policies for R&D (Fig. 3). The birth of biotech industry, the outsourcing of industrial R&D to academia, to publicly funded science, and to small and medium enterprises are part of the current context and of the globalization process [94]. Together, these changes result in the push for rapid development of marketable products. The long-term, public funding of science deemed as of strategic interest between 1945 and the 1980s is now massively replaced by a "short-termist" view of investment [96]. As stem cells come to center stage as likely tools for novel approaches to medicine, governments and the private sector alike demand short-term return of their investment in R&D in the guise of marketable products. In a financial rather than industrial business model, the approach itself, or the hope itself (rather than a tangible object such as an effective therapy) become the marketed commodity [97]. The marketing of immature approaches to therapies [98,85] then generates societal, medical and scientific issues. The societal issues are exemplified by the frequent use of "MSCs" in the despicable "stem cell tourism" around the world [99], and by the push to legalize their marketing ahead of any proof of efficacy [100]; medical issues, by the resurgence, particularly among some academic physicians, of a prescientific empirical approach to medicine, which had taken centuries to overcome [101]. At this time, almost 400 underpowered clinical trials around the World, mostly in the East and the Caribbean, use intravenous MSCs in patients with severe diseases that are not only without a cure, but also without a chance of being cured by intravenous infusions of MSCs. Scientific issues, lastly, are exemplified by the diffusion of scientifically feeble and medically ungrounded notions, which permeate a vast scientific literature and do not spare even the most prestigious venues for publication. Bone stem cells ("MSCs") cannot cure autism or stroke as claimed. History records major examples of how ideology (religious or political) can disseminate non-scientific misbeliefs and hold them in the face of, or against, scientific evidence. The power of rising commercial interests to do the same is a novelty of this stretch of history. At a glance, it seems to contradict the historical alliance of economic development and rigorous science as a source of technology, medical technology included. In economics, however, it is a known fact (Gresham's law) that "bad money drives the good one out".

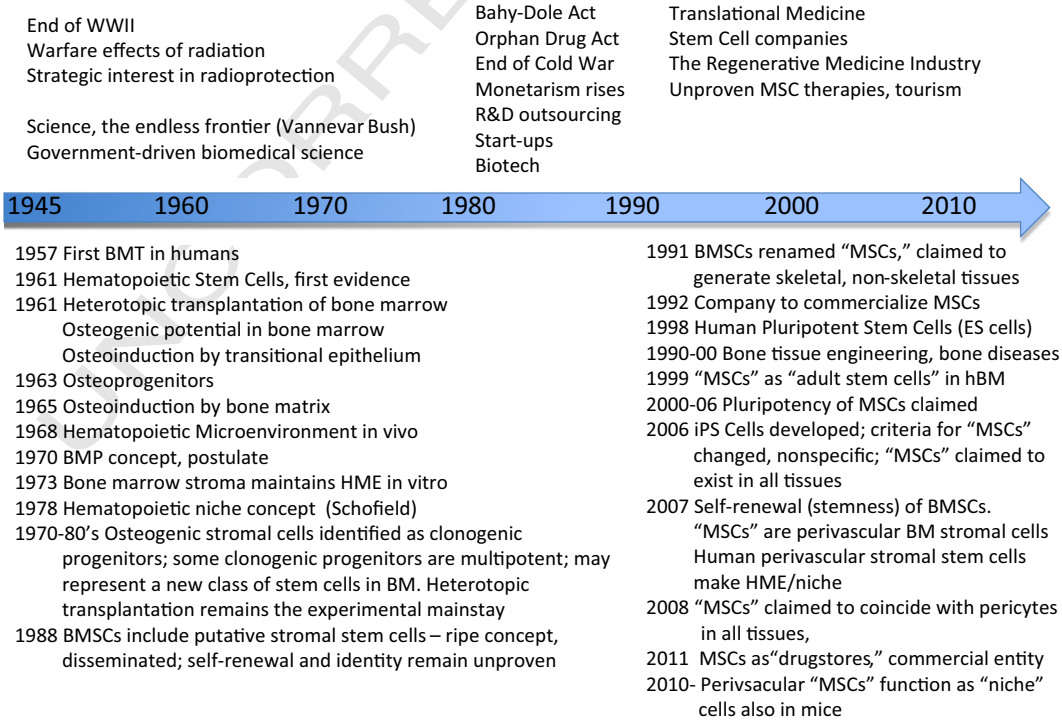

Fig. 3. Diagram briefly summarizing the main achievements and shifts in paradigm over the last 70 years in the science of stem cells and bone, and in the general political and economical climate in the West, reflected in scientific policies.

Please cite this article as: Bianco P, Stem cells and bone: A historical perspective, Bone (2014), http://dx.doi.org/10.1016/j.bone.2014.08.011

The history of stem cells in bone is deeply intertwined with the history of the world over the last 70 years. Between 1945 and 1980s, it provides the most impressive example of how the paradigm of the time, sculpting a strategic role of science and of its public funding, worked productively: bone marrow transplantation, hematopoietic stem cells, and skeletal stem cells are all the legacy of those decades, and of the post-War view of science and medicine in society. Between the 1980s and present day, a "historical" look at stem cells in bone gives a glimpse on the effects on science and science policies of changing commercial interests, which tend to replace and displace a strategic (beyond the military sense) role for science in society in peacetime. Still, the history of stem cells in bone is replenished, throughout the 70 years, with major intellectual, scientific and medical advances. As articles in this issue show, more advances in biology, medicine and technology in a number of areas from cancer to genetic diseases are in sight, making science itself more viable and creative than the frame of policies in which it has lived in the last two decades.

Acknowledgments

Personal work mentioned in this article was supported by the Telethon Foundation (Grant GGP09227), the MIUR, Fondazione Roma, Fondazione Institut Pasteur-Cenci Bolognetti, the Ministry of Health of Italy, the EU (Plurimes consortium) and Sapienza University of Rome.

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Please cite this article as: Bianco P, Stem cells and bone: A historical perspective, Bone (2014), http://dx.doi.org/10.1016/j.bone.2014.08.011

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