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Review

Non-viral gene activated matrices for mesenchymal stem cells based tissue engineering of bone and cartilage

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ABSTRACT

Recent regenerative medicine and tissue engineering strategies for bone and cartilage repair have led to fascinating progress of translation from basic research to clinical applications. In this context, the use of gene therapy is increasingly being considered as an important therapeutic modality and regenerative technique. Indeed, in the last 20 years, nucleic acids (plasmid DNA, interferent RNA) have emerged as credible alternative or complement to proteins, which exhibited major issues including short half-life, loss of bioactivity in pathologic environment leading to high dose requirement and therefore high production costs. The relevance of gene therapy strategies in combination with a scaffold, following a so-called "Gene-Activated Matrix (GAM)" approach, is to achieve a direct, local and sustained delivery of nucleic acids from a scaffold to ensure efficient and durable cell transfection. Among interesting cells sources, Mesenchymal Stem Cells (MSC) are promising for a rational use in gene/cell therapy with more than 1700 clinical trials approved during the last decade. The aim of the present review article is to provide a comprehensive overview of recent and ongoing work in non-viral genetic engineering of MSC combined with scaffolds. More specifically, we will show how this inductive strategy can be applied to orient stem cells fate for bone and cartilage repair.

1. Introduction

Tissue engineering (TE) is in a phase of rapid development as a new multidisciplinary healthcare biotechnology domain, which promises to change medical practice profoundly. Its founding paradigm is to elicit the regeneration of injured tissues and organs instead of replacing them with inert artificial implants [1]. It is now well-established that the most promising approach to obtain full tissue regeneration is a pertinent combination of cells, biomaterial scaffolds and biochemical/biophysical cues adapted to the tissue physiology [2].

Various cell types have been investigated for tissue regeneration. Differentiated autologous cells were used in a wide range of applications to replace defective tissue, as in cartilage [2,3], cardiac tissue [5] or skin wound healing [6]. However, the difficulty to massively expand these cells while keeping their differentiated state and properties *in vitro* and *in vivo* has appeared as a major limitation to clinical development. Pluripotent stem cells, such as embryonic (ESC) or induced pluripotent (IPS) stem cells are currently investigated and proved their efficiency to differentiate into, for example, chondrocytes [7]. Nevertheless, major obstacles, including allogeneic rejection and teratoma formation, as well as complex differentiation protocols still hinder their translational potential. By contrast, advances in the understanding of adult mesenchymal stem cells (MSC) have revolutionized tissue engineering, particularly within the field of skeletal regenerative medicine thanks to their great self-renewal ability, their aptitude to differentiate into three major lineages (chondrocytes, osteocytes and adipocytes [8]) as well as their secretive properties [9–12].

The use of scaffolds in TE was initially based on the notion that it would both favor cell attachment, survival, and would also act as a template for neo-tissue formation. From this perspective, some requirements to build the material were therefore proposed for efficient *in vivo* implantation [13]: (i) three-dimensionality and high porosity for cell/tissue infiltration and growth as well as transport of nutrients, oxygen and metabolic waste; (ii) biodegradability or bioresorbability with a controllable degradation and

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resorption rate; (iii) facility to be processed into a variety of shapes and dimensions. Moreover, the scaffold must present (iv) a suitable surface chemistry for cell attachment, survival and differentiation and (v) convenient mechanical properties to match those of the targeted tissues. To answer these issues, biomaterials with a wide variety of compositions have been used for tissue engineering: organic, inorganic or a combination of both, depending on the requirements of the application [14–16]. Various processing methods have been used to elaborate the scaffold as a dry material or hydrogel [13], [17]. The resulting structural features of these scaffolds were shown to greatly influence stem cells differentiation: mechanical properties [18], porosity [19] as well as cell seeding density [20] could induce specific lineage differentiation by affecting cell-cell and cell-scaffold interactions. Stem cells behavior (viability, adhesion, proliferation, migration) is also strongly affected by the chemical composition [21] and micro- or nano-architecture [22,23], of the scaffold. Indeed, when seeded onto polymers reinforced with inorganic materials like hydroxyapatite [24] or glass ceramics, stem cells expose enhanced osteogenesis potential. The choice of the material is therefore *in fine* strongly determined by the final clinical application. In the prospect of *in vivo* implantation, scaffolds have been designed to serve as reservoirs for proteins, with finely tuned formulation to allow controlled and sustained delivery as well as protection of these crucial biochemical cues to orient MSC differentiation [25–28].

In parallel to proteins or peptides, the use of nucleic acids to orient MSC into a particular lineage differentiation appeared as a complementary and powerful strategy [29]. One of the first strategy that was investigated is to transform stem cells by introducing a therapeutic gene that will induce the expression of soluble factors able to direct cell differentiation towards a specific lineage mainly relying on pDNA use [30], or more recently with chemically modified (cmRNA) [31]. Following this approach, the superfamily of transforming growth factors TGF-β, also including bone morphogenetic proteins (BMP), represents the main targets to influence MSC differentiation into osteocyte or chondrocyte [32–37]. A different approach, also based on gene induction, relied on the induction of the expression of transcription factors or transducing proteins which will direct cells fate by activating intracellular pathway [38–40]. In parallel, thanks to the increased knowledge concerning MSC biology, numerous RNA interference (RNAi) molecules have been evaluated to influence MSC differentiation towards osteogenic (siRNA [41–46] and miRNA [47–56]) or chondrogenic lineages (siRNA [57,58], and miRNA [59–63]).

Whatever the target, the success of the approach implies high transfection efficiency associated with high cells survival rate. Therefore, an efficient NA vectorization strategy has to be found, as nucleic acids (NA) are negatively charged hydrophilic molecules which therefore do not easily cross the lipophilic and negatively charged cell membrane. Viral vectors proved to be relatively efficient for gene transfer [64,65], and are widely used for cell transformation *in vitro*. However, safety concerns have been raised regarding their use for gene delivery *in vivo*, especially because of the immune response of the host, possible mutagenesis, and lack of specificity of transgene delivery in addition to high production costs [66]. In this context, non-viral vectors are often preferred for *in vivo* use because they are safe, easy to handle and cost-effective, the main drawback remaining their low and transient transfection efficiency [67]. Nevertheless, we will try to illustrate in this review that these drawbacks could be circumvented by associating non-viral vectors with scaffolds. This combinatorial strategy is generally termed “Gene Activated Matrix” (GAM). The first *in vivo* proof of concept of a GAM in bone repair application was established by Fang JM et al. in 1996 [68] using a collagen sponge impregnated with an association of 2 plasmids encoding BMP-4 and the parathyroid hormone fragment, acting synergistically *in vitro*, and leading to new bone formation *in vivo*. This study demonstrated for the first time that fibroblasts implanted in bone can be genetically manipulated *in vivo* to produce proteins capable of inducing bone growth. Since then, various combinations of scaffolds and nucleic acids (vectorized or not) have been used for cartilage and bone repair, associated or not to various cell types.

In this work, we will particularly describe the most recent NA vectors used to transfect MSC. The crucial influence of both scaffolds structural properties and interactions between vectors and scaffolds on transfection rate will be particularly discussed. Finally, the pertinence of this various parameters will be illustrated through a description of the most recent and promising GAM applied to orient MSC fate for bone and cartilage repair.

2. Progress in non-viral vectorization of nucleic acids into MSC

Depending on the strategy used for gene therapy (plasmids, RNA interference), the targeted cellular compartment will be different, *i.e.* cytoplasm or nucleus, leading to different trafficking pathways requirements. Concerning non-viral vectors formulation, a large majority of synthetic vectors relies on the use of cationic polymers or lipids interacting with the NA through complexation of their negatively charged phosphate groups. Recently, specific efforts have been directed towards taking into account the structural features of the nucleic acids used and their trafficking pathways in the design of a specific vector [69].

Primary cells like MSC are reputed difficult to transfect [70] this stimulated an active research to develop original, reproducible, safe and efficient systems suited for NA protection and vectorization [71–73]. In 2006, Mac Mahon et al. described that only 25% of rat MSC were transfected with Lipofectin®, compared to 70 and up to 95% for adenovirus and lentivirus respectively [74]. Since then, great work has been made to enhance this transfection rate. We will here try to draw a non-exhaustive list of the most recent progress in non-viral vectors development for MSC in comparison to previously existing systems (Table 1).

2.1. Lipid based systems for MSC transfection

Numerous lipid-based non-viral vectors have been investigated and commercialized for *in vitro* MSC transfection, with various transfection efficiencies depending on their cargo. For instance, Lipofectin®, Lipofectamine2000®, Metafectene® and Lipofectamine Plus® led to transfection rates varying from 50% to 20% for pDNA [74–76]. By contrast, higher efficiencies were obtained for the vectorization of siRNA with Lipofectamine® 2000 (98%) [75] or TransIT®-TKO (95%) [77], although associated with a rather high cytotoxicity (30–45% cell death). Other commercial lipidic carriers specifically designed for siRNA vectorization showed lower uptake rates of 77% for RNAiFect®, 27% for GeneEraser™, 8% for RiboJuice™, and even 0.3% for siPort Lipid [75]. Recently, gene silencing strategies using microRNA (miR or miRNA) emerged with the aim to expand the therapeutic potential of human MSC (hMSC), as this powerful and versatile molecules were described to play an important role on MSC fate [78]. Lolli et al. recently described that the silencing of miR-221 promoted chondrogenesis in 3D MSC pellets cultured without TGF-β supply, highlighting the strong potential of using miRNA as a tool to orient MSC differentiation [79]. By transfecting hMSC *in vitro* with Lipofectamine® RNAiMAX, they demonstrated that miR-221 silencing was superior to 95%.

Focusing on vector development, non-phospholipid liposomes (stereosomes) with single-chain amphiphiles and high content of sterols (stearylamine and cholesterol) have been recently evaluated