Transgene expression is difficult at the best of times. However, the use of minicircle DNA can make this process much easier, as they allow better nuclear entry. Progressive research in gene and cell therapy, as well as DNA vaccination, requires safe pharmaceutical vectors. The former, in particular, is on the rise, with a huge potential for treating inherited disorders. However, the advances in this area depend on improved vector designs, which enable the safe delivery of therapeutics into target cells (1). The novel transfer vectors should be direct, tissue-specific, stable and highly expressive. They have to be minimally toxic, regarding anti-vector immune responses, and should not be integrated into the host genome (2). Without integration, the toxicity is low, but this also means that long-term expression – and thus therapeutic effect in mitotic cells – may not occur because of progressive loss of vector DNA (2).

Usually, viral and non-viral vectors based on plasmid DNA are used for the transfer of genetic information into the target cells. Plasmids encode these on the intracellular production of therapeutic virus particles (3). Clinical use of plasmid DNA for gene or cell therapy, as well as genetic vaccination, has been in an inferior position to viral methods, but has made significant progress in recent years (4,5).

**Challenging Transfers**

One limiting factor in transgene expression is the transfer process into the cytoplasm and nucleus, as the size of the DNA is crucial (6,7). The smaller it is, the better the nuclear entry. The differing proportions between two otherwise identical plasmids, such as monomer and dimer, show the influence of this procedure (8).

Using minicircle (MC) DNA for the process holds a great advantage due its minute size (6). The contained bacterial backbone of conventional plasmids with resistance sequences, origin of replication and immunogenic cytosine-phosphatidyl-guanine (CpG) motifs is not necessary for gene expression – the structure of a typical plasmid is shown in Figure 1. The backbone could also be a potential risk factor for safe clinical application and leads to decreased gene transfer and expression (9). To avoid this disadvantage, such sequences are removed by the MC technology, and the size of the DNA is simultaneously reduced.

Kobelt et al demonstrated that green fluorescent proteins – alongside luciferase and lacZ-encoding MCs – transferred into human melanoma and colon carcinoma cell lines by lipofection or electroporation lead to enhanced transgene messenger RNA transcription compared to their corresponding plasmids (9). Furthermore, a higher gene transfer rate was shown through fluorescence-activated cell sorting analysis (9). Therefore, MCs in reproducible high-quality are an efficient alternative to conventional plasmids and, due to their non-integration, they provide an improved safety profile in hepatic gene therapy, for example (2).

**CAR T-Cells**

A recent study evaluated the application of MC DNA in cancer immunotherapy using chimeric antigen receptor (CAR) T-cells. Currently, these are modified by gamma-retroviral or lentiviral vectors for efficient immunotherapy to express CAR. Besides low transfection rates, genotoxicity of viral vectors is a safety issue. Monjezi et al has shown that the use of non-viral Sleeping Beauty (SB) transposition of CAR genes (derived from MCs) is more efficient and less toxic to T-cells than current plasmid-based approaches (10). Therefore, the non-viral SB transposition provides an effective and safe gene transfer strategy and represents a preferable alternative to viral vectors (10). Due to the higher transposition rate and lower toxicity of the novel MC-based approach, an induction with feeder cells is not required to provide therapeutically relevant CAR-T-cell numbers (11).
Another application of MC DNA is the manufacturing of adeno-associated viruses (AAV). So far in manufacturing, backbone sequences, such as the resistance genes of conventional plasmids, can be transferred into AAV capsids through false packaging events, also known as retro-packaging, thus enabling their reach of the target organism. Current studies show distinct evidence that AAV vector production based on MC DNA avoids the incorrect packaging of bacterial sequences in the capsids (12).

Meeting Regulatory Requirements

Antibiotic resistance genes are often used as selection markers on plasmid DNA vectors in bacterial hosts, as are the other sequences that allow selection or maintenance, which show the same disadvantages: they represent a biosafety risk for application in gene therapy. Therefore, the EMA adapted their guidelines for medical gene transfer products, avoiding selection markers such as resistance against antibiotics (13).

As aforementioned, the MC technology removes these resistance genes and the prokaryotic origin of replication as well, resulting in a natively supercoiled monomeric molecule that is almost free of any bacterial sequences (12). In conclusion, MCs are a promising tool for applications in gene and cell therapy, vaccination or as a starting material for viral vector manufacturing.

References

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