Supplementary Materials: mRNA as a Tool for Gene Transfection in 3D Cell Culture for Future Regenerative Therapy

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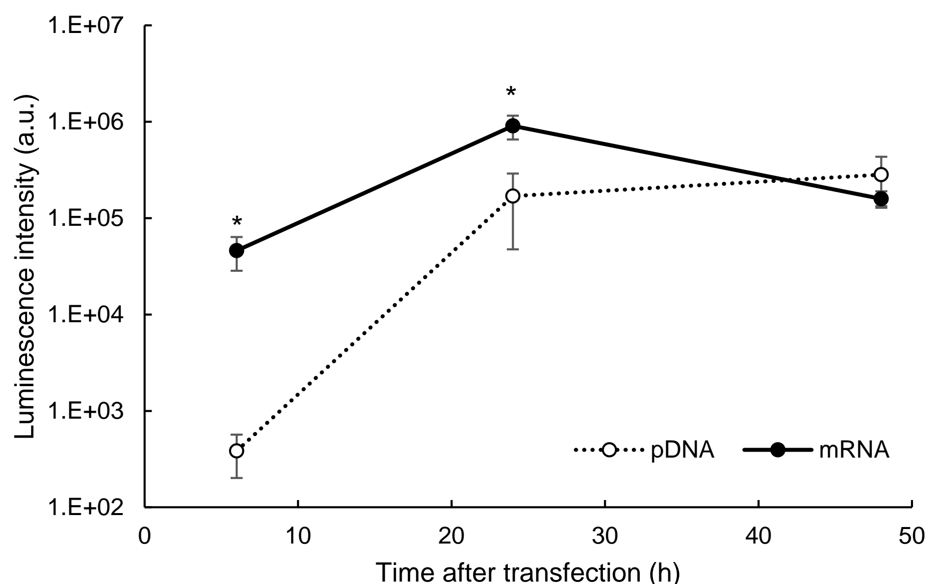

Figure S1. Time dependent profile of transfection efficiency. MSC spheroids were transfected with mRNA and pDNA encoding *Gaussia luciferase* (*GLuc*). A coding sequence of *GLuc* in a pCMV-GLuc control vector (New England BioLabs, Ipswich, MA, USA) was inserted into pSP73 vector (Promega, Madison, WI, USA) possessing a 120 bp poly A/T sequence for preparation of mRNA by *in vitro* transcription, and into pCAG-GS vectors (RIKEN, Tokyo, Japan) for *GLuc* pDNA construction. Note that both mRNA and pDNA have an identical coding sequence of *GLuc* (Genbank: MO429382.1). Subsequently, MSC spheroids cultured in 96-well micropatterned plates were transfected with *GLuc* mRNA or pDNA complexed with PAsp(DET) at N/P ratio of 10, as described in Section 2.3. At indicated time point, culture medium was collected for luminescence measurement using Renilla Luciferase Assay System (Promega) and a GloMax-Multi+ Detection System (Promega, Madison, WI, USA), followed by replacement of the medium with the fresh one. $n = 4$. Data are presented as mean ± standard error of the mean. Statistical analysis was performed by unpaired 2-tailed Student's *t*-test. * $p < 0.05$ vs. pDNA.