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Surgery for the 21st Century: Biomimetic Nanotechniques and Extracellular Vesicles

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Introduction

"Surgery: the branch of medical practice that treats injuries, diseases, and deformities by the physical removal, repair, or manipulation of organs and tissues."

Nothing in the definition of "surgery" limits it to removing tissue (tumors), introducing tissue (transplants), implanting devices, or involving techniques using traditional instruments such as a scalpel, laser ablation, or radiosurgery.

The 21st century surgeon will increasingly use techniques not usually considered "surgical" as our knowledge of disorders of various tissues increases and our surgical armamentarium expands.

The COVID-19 pandemic engendered intensive efforts to develop a vaccine quickly. Fortunately Moderna and Pfizer-BioNTech had been working on a biomimetic technique to enable the introduction of messenger RNA (mRNA) into cells in order to disrupt the multiplication of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The technique involved development of a lipid nanoparticle vesicle containing the mRNA, thus allowing mRNA to be delivered from the injection site to the target cells without degradation (Figure 1).[1,2]

mRNA vaccines are biomimetic examples of extracellular vesicles (EVs) – one major form of communication between cells throughout the body. EVs, and exosomes (one type of EV) in particular, will become an essential part of traditional surgical techniques, from diagnostic biopsy to tumor eradication to organ regeneration. Surgeons can take a leading role in moving EVs from bench to bedside.

Extracellular Vesicles (EVs) – the Body's Internet

The title of a recent article says it all:

"A brief history of nearly EV-erything – The rise and rise of extracellular vesicles"[3]

Chronologically, the history of EVs largely paralleled the development of the internet. The terms "microparticles" and "matrix vesicles" were used in the 1960s.[3] In 1974 the process "*fusion of the outer or limiting membrane of the multivesicular body with the apical plasma membrane might lead to the release of the vesicles contained within the structure into the luminal space*" was described and the term "extracellular vesicle" coined.[4] The vesicles produced by this process became known a decade or so later as "exosomes"; which were initially thought to be a cellular mechanism for disposal of "obsolete membrane proteins".[3,5]

The following decade saw the beginning of appreciation for the many roles of EVs, from markers for brain and cardiac ischemia to potential anti-tumoral vaccines.[3] The period since the year 2000 has seen an explosion of research on EVs: from roughly 1000 articles in the period 1985 to 2000 to more than 4000 articles over the following decade.[6] A problem in the field has been the lack of standardization in terminology; this challenge along with the burgeoning interest in EVs led to the formation of the International Society for Extracellular Vesicles (ISEV) in 2011. From the year 2000 until 2018, more than 500 US patents were granted that included terms referring to EVs and more than 30 clinical trials involving EVs either as diagnostics or therapeutics were conducted (primarily in the field of cancer biology).[3,6]

Like the internet for communication among the world's citizens, EVs have come to be understood over the past two decades as a major source of communication among the cells of the body. Going forward, the surgeon's role in biopsy for diagnosis, eradication of diseased tissue, and regeneration of degenerated organs will depend on an understanding of EVs and their potential for what are at present (but perhaps not for long!) conditions treated by traditional surgical techniques.

EVs – Packets of Information

EVs come in various sizes and functions (Figure 2).[7] Large EVs range in size from 200 nm (nanometers) to 1 mm (micron – about 1/10th the diameter of a red blood cell). Supermeres, the smallest EVs described to date, may be as small as 10 nm or less. In Figure 2, small EVs are commonly called exosomes. Exosomes are secreted by all cells. EVs with other functions – most notably oncosomes released by cancer cells and apoptotic bodies originating from membrane disintegration after apoptosis – may be as large as 10 mm.

A schematic of exosome biogenesis and structure is given in Figure 3.[7] There are three stages (Figure 3A): (1) an endocytic vesicle forms from the cell's plasma membrane; (2) the endocytic vesicle matures into a multivesicular body (MVB); (3) the MVB (if not degraded by fusion with a lysosome) fuses with the plasma membrane to release the exosome. As demonstrated in Figure 3B, the cargo (e.g. lipids, proteins, nucleic acids, RNAs and DNAs) of the exosome depends on the cell of origin (and that cell's status at the time of exosome formation); the exosome membrane is a bilayer composed of lipids (e.g. cholesterol, phospholipids, ceramide sphingolipids) as well as various proteins that function either as protective antigens or as targeting/adhesion molecules.[7] An interesting relationship between neurotransmitters and exosome release from microglia in the nervous system has been demonstrated: serotonin stimulates exosome secretion from microglial cells.[8]

Figure 4 illustrates many of the characteristics and advantages of exosomes for diagnosis and treatment.[7] Two potential applications of exosomes are addressed in the next section: (1) optimization of inflammatory response following injury, and (2) neurorepair for spinal cord injury.

EVs – EV-erywhere!

Recent studies have highlighted a plethora of potential therapeutic applications of exosomes – from skin wound healing to autoimmunity to cardiac and intervertebral disc repair to tumor treatment and COVID-19 mRNA vaccines. A major issue in recovery from tissue injury is optimization of the inflammatory response. Both microglia in the central nervous system and macrophages in the rest of the body undergo massive recruitment following injury, the early phase of the response being largely pro-inflammatory with the later phase being largely anti-

inflammatory. Macrophages and microglia during the pro-inflammatory phase have been termed "M1", during the anti-inflammatory phase "M2". An area of intense research aims at polarizing macrophages/microglia to M2 rather than M1 state or phenotype.[9,10] Our understanding of the effects of M1 and M2 microglia has already become incredibly complex, although clearly incomplete to date.[10]

Exosomes derived from mesenchymal stem cells (MSCs) are a major research topic at present. One reason is that exosomes derived from MSCs can modulate microglia from M1 to M2 phenotype (Figure 5).[11] Exosomes can be carriers of microRNAs (miRNAs); miRNAs are particularly important for diagnosis and treatment of various disorders, including stroke (Figure 6).[12,13] The nature and diverse functions of miRNAs have been summarized:

"miRNAs, small non-coding RNAs, average 22 nucleotides in length. Most miRNAs
are transcribed from DNA sequences into primary miRNAs… miRNAs are critical for
normal animal development and are involved in a variety of biological processes.
Aberrant expression of miRNAs is associated with many human diseases… miRNAs
are secreted into extracellular fluids. Extracellular miRNAs are potential biomarkers
for a variety of diseases; they also serve as signaling molecules to mediate cell-cell
communicaitons."[13]

A major challenge for exosomes derived from MSCs is targeting the exosomes to the tissue (or injury site) to be addressed. This is similar to the problem of drug targeting in general: one wants the effects to be maximal with the side-effects (due to ineffective targeting) to be minimal. One targeting technique is incorporating the membrane from cells that typically target the tissue or injury site – such as macrophages and microglia – into the membrane of the exosome (or exosome-like nanoparticle carrying the desired therapeutic cargo).[7,14]

One example of macrophage membrane-fused exosome-mimetic nanovesicles (MF-NVs) for the treatment of spinal cord injury is given in Figure 7.[15] Membranes isolated from macrophages were fused with MSC; NVs containing RNAs and proteins similar to MSC-derived exosomes, formed by porous membrane extrusion, incorporated the membrane proteins from the macrophage – enhancing delivery of the exosome-mimetic therapeutics to the spinal cord injury site. The results of this study are illustrated in Figure 8.[15] The improvement 28 days after spinal cord injury – on direct imaging, immunohistochemical staining, and Basso Mouse Scale (BMS) functional testing – is quite impressive.

Conclusion

Although surgeons have been innovative with more precise and minimally invasive traditional surgical techniques, in the coming decades (more likely, the coming years!) it will be increasingly obvious that surgeons need to adopt surgical techniques at the cellular level. Cutting and suturing will be accomplished at the level of the cell, using techniques we all can develop in concert with our colleagues in biochemistry and genetics.

Figures

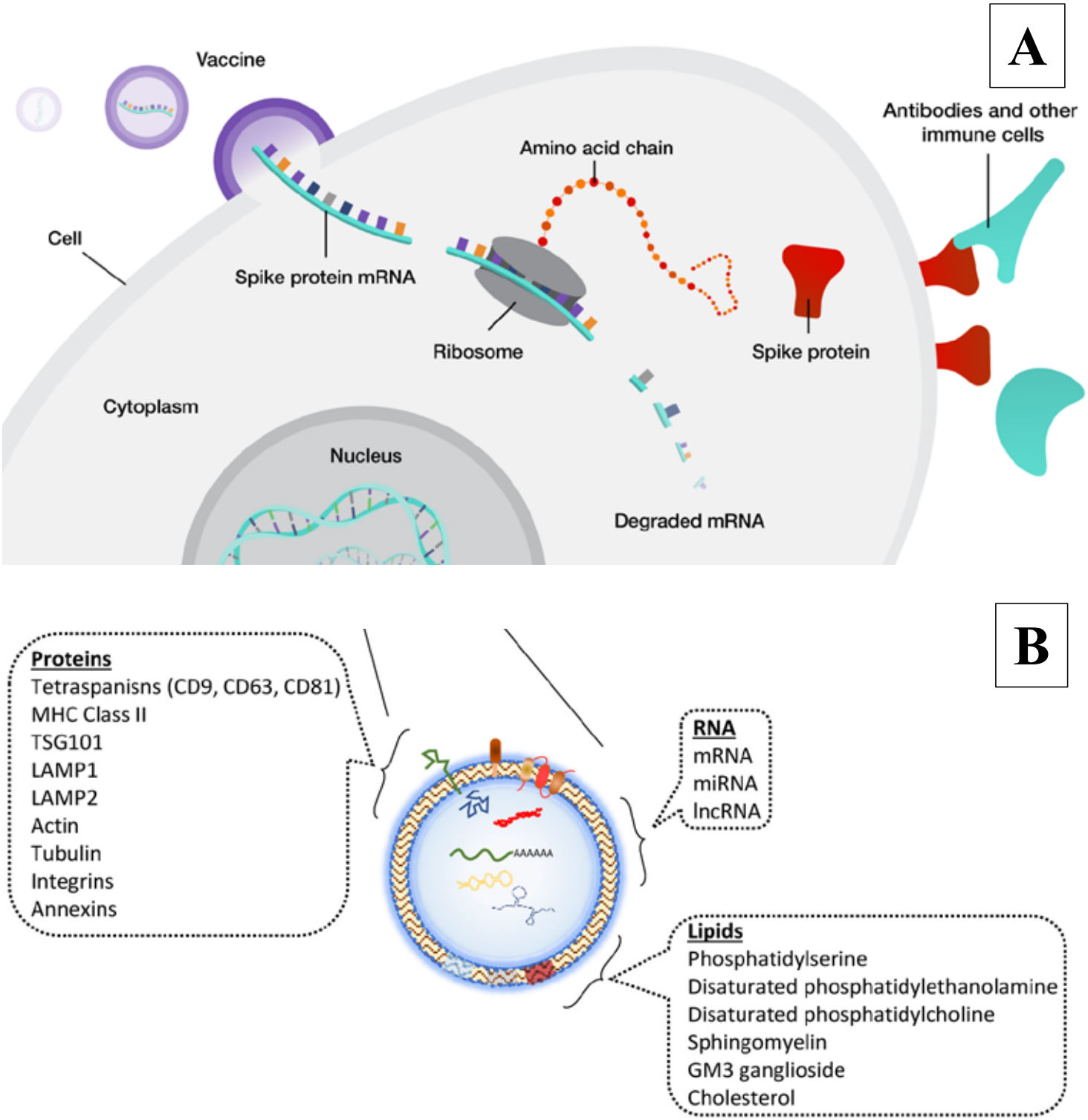


Figure 1: A: Schematic of mRNA in lipid nanoparticle entering cell to evoke an immune response against the viral spike protein (ref #1 open access). **B**: Structure of an exosome (one type of extracellular vesicle) – similar to the COVID-19 mRNA vaccine lipid nanoparticle. (ref #2 open access)

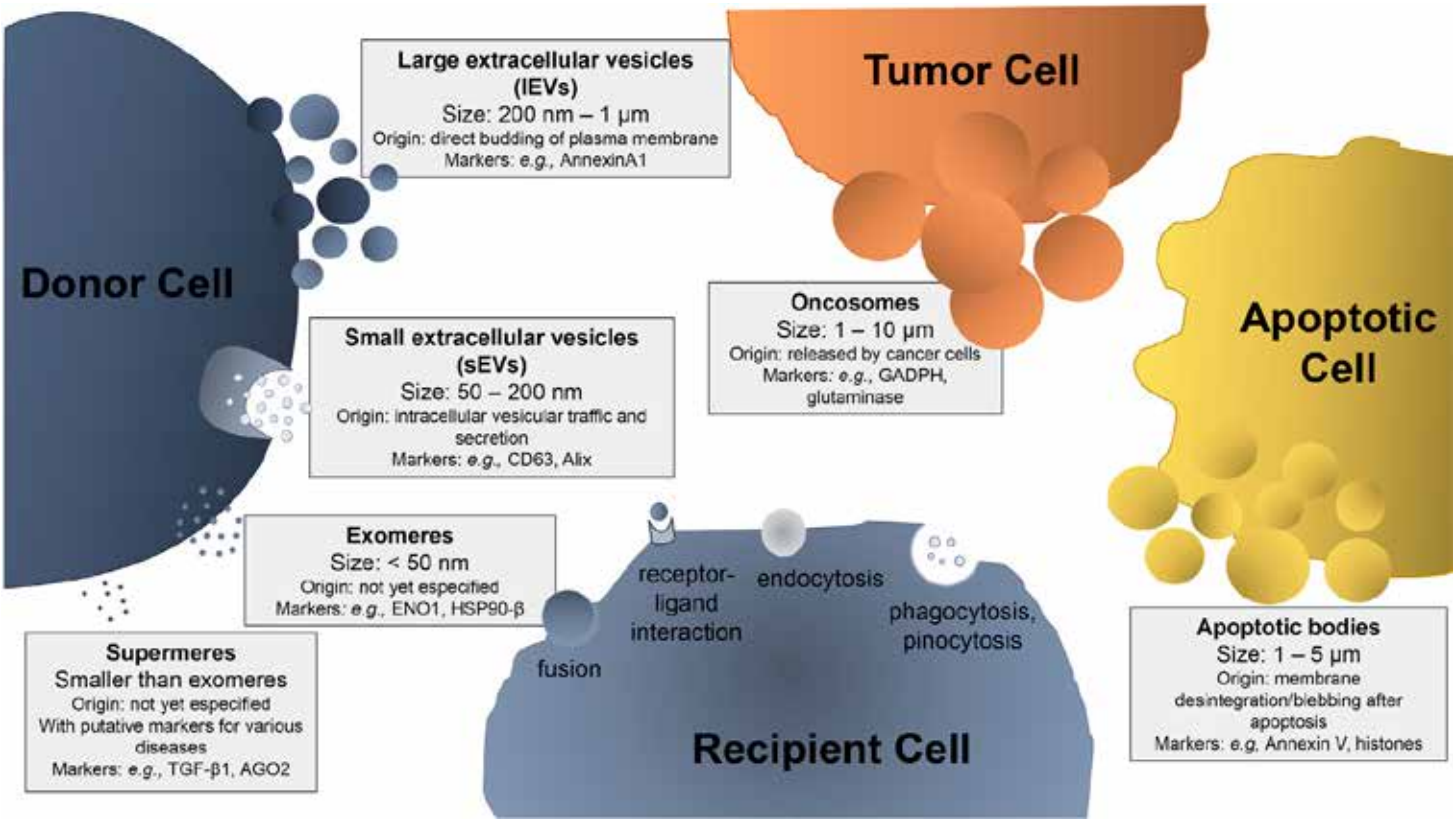


Figure 2: Schematic of EV subtypes, sizes, and characteristic markers. (ref #7 open access)

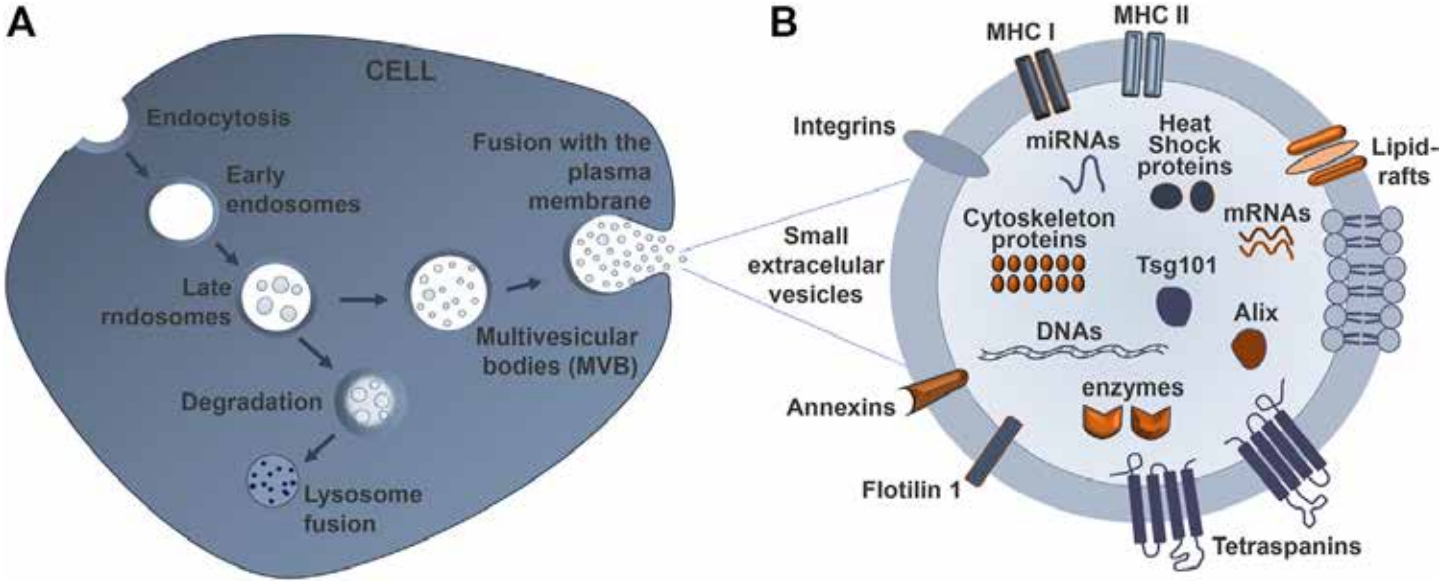


Figure 3: Schematic of exosome biogenesis (**A**) and its typical structure (**B**). (ref #7 open access)

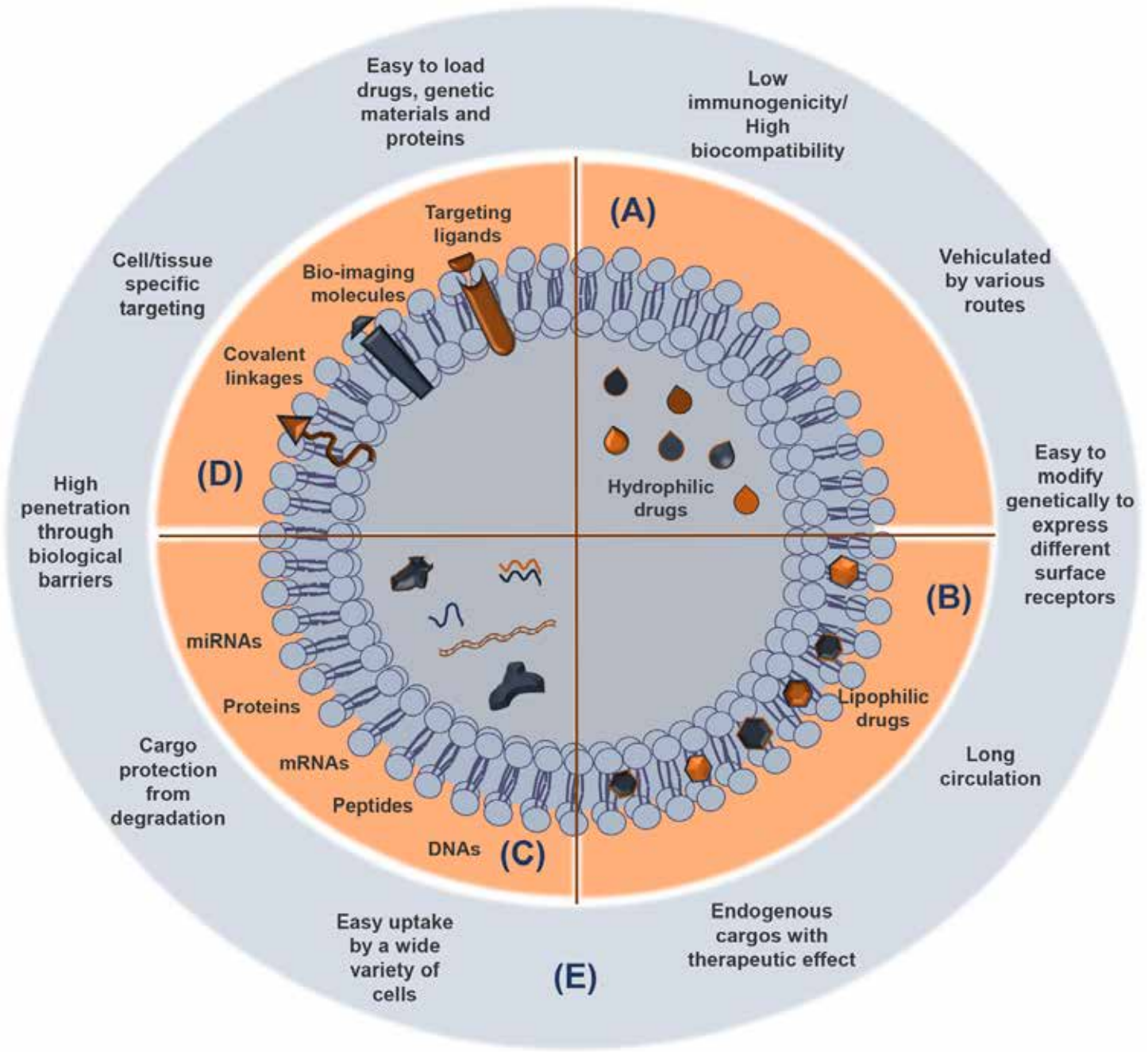


Figure 4: Exosome cargoes and various delivery systems for molecules. **A**: Hydrophilic molecules in aqueous compartments. **B**: Lipophilic molecules entrapped in the lipid bilayer. **C**: Release of proteins, peptides, and genetic material from exosomes. **D**: Targeting compounds, bio-imaging molecules, and covalent linkages on the exosome surface enhance delivery capability. **E**: Major advantages of exosomes. (ref #7 open access)

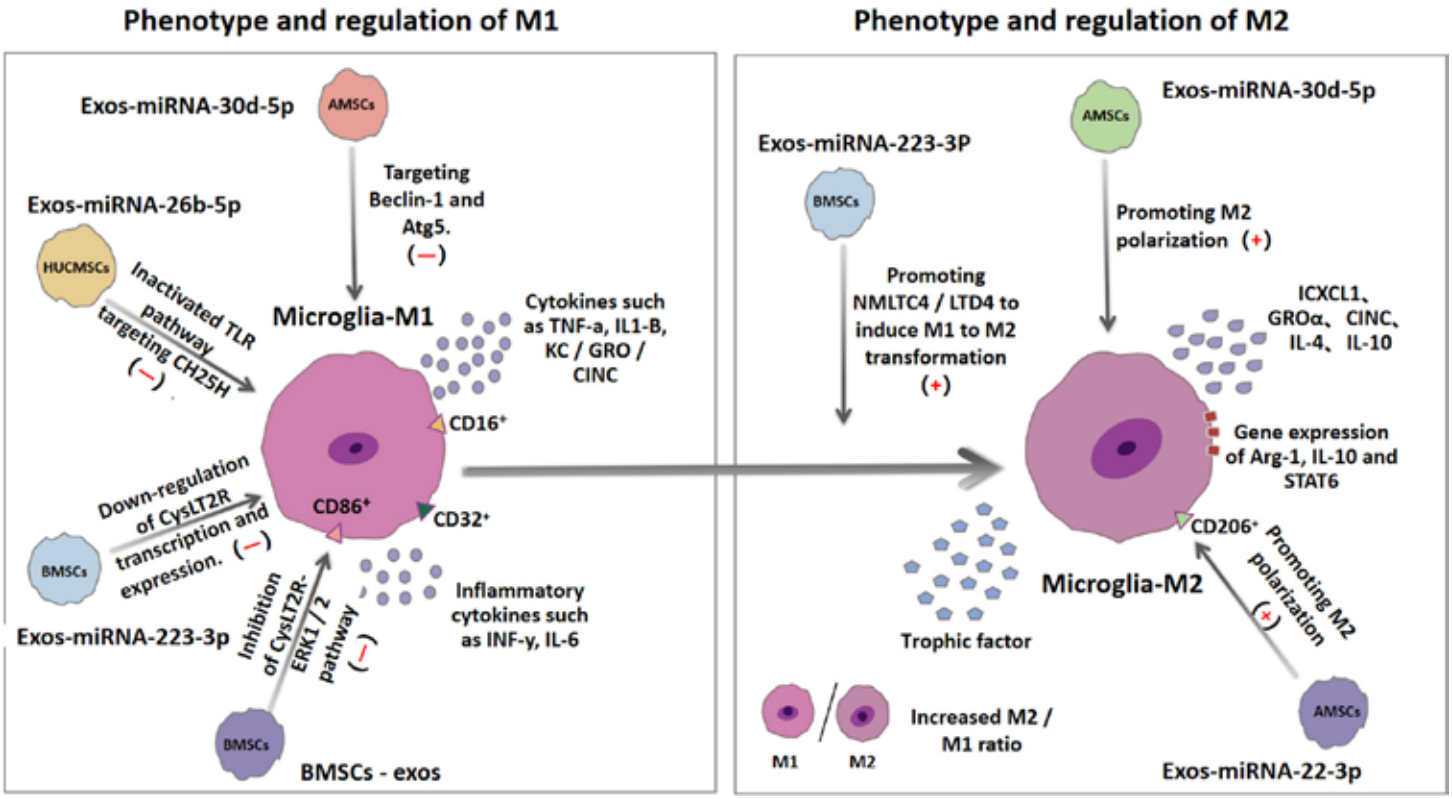


Figure 5: Exosomes containing microRNAs (miRNAs) derived from mesenchymal stem cells can up- or down-regulate the production of M1 and M2 microglia, thereby reducing the inflammatory response and secondary tissue damage after central nervous system ischemia. (ref #11 open access)

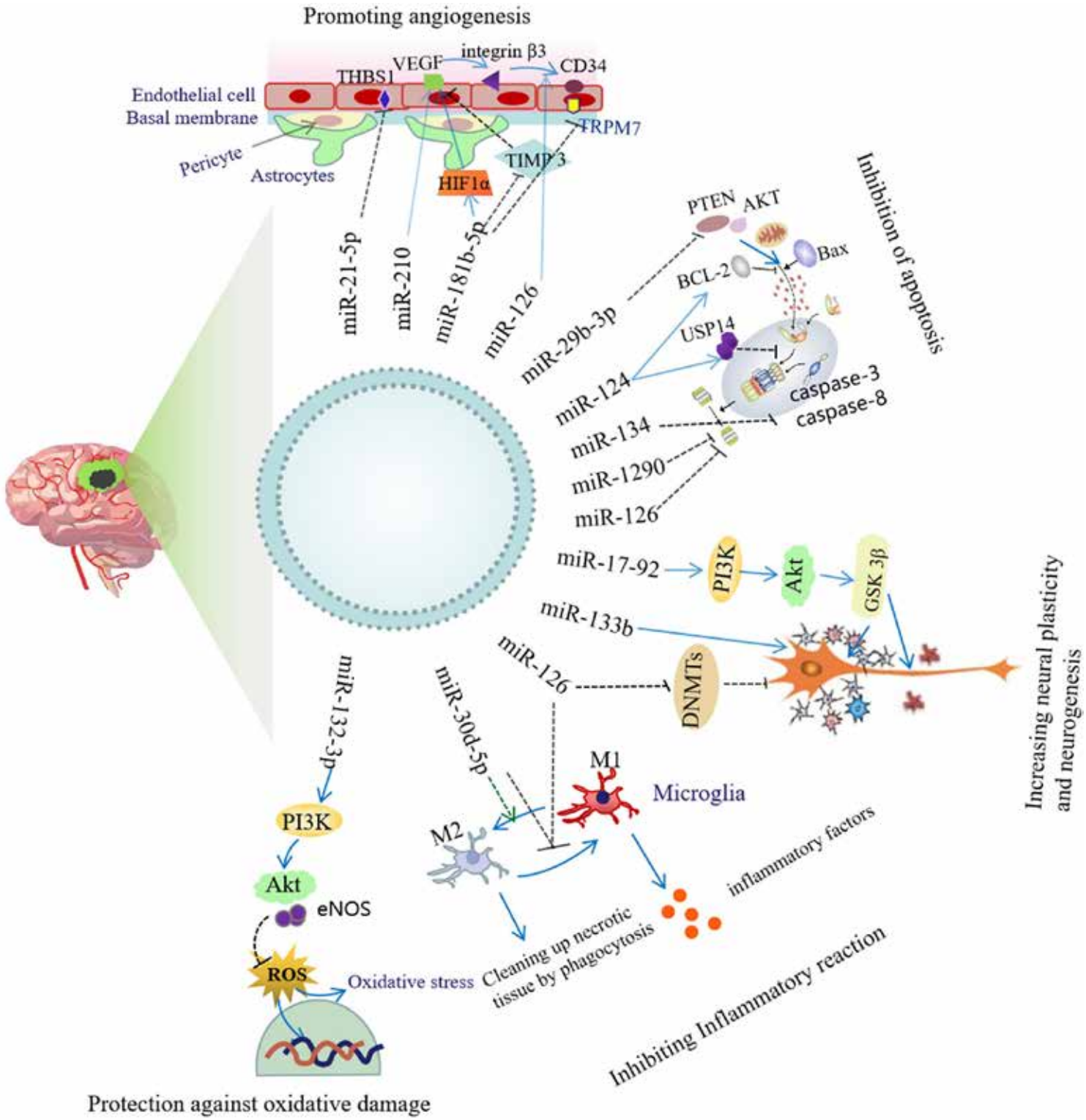


Figure 6: Neuroprotective mechanisms of exosomal miRNAs.
All abbreviations are provided in ref #12. (ref #12 open access)

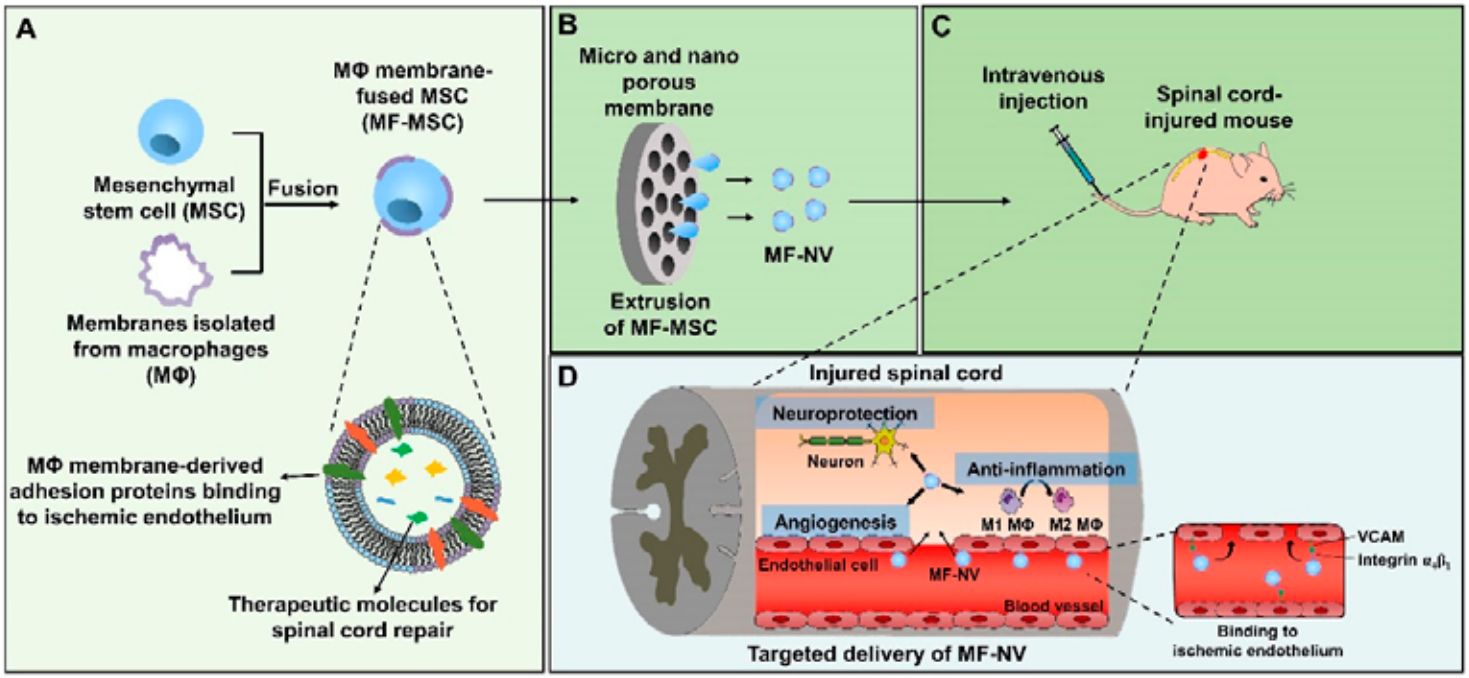
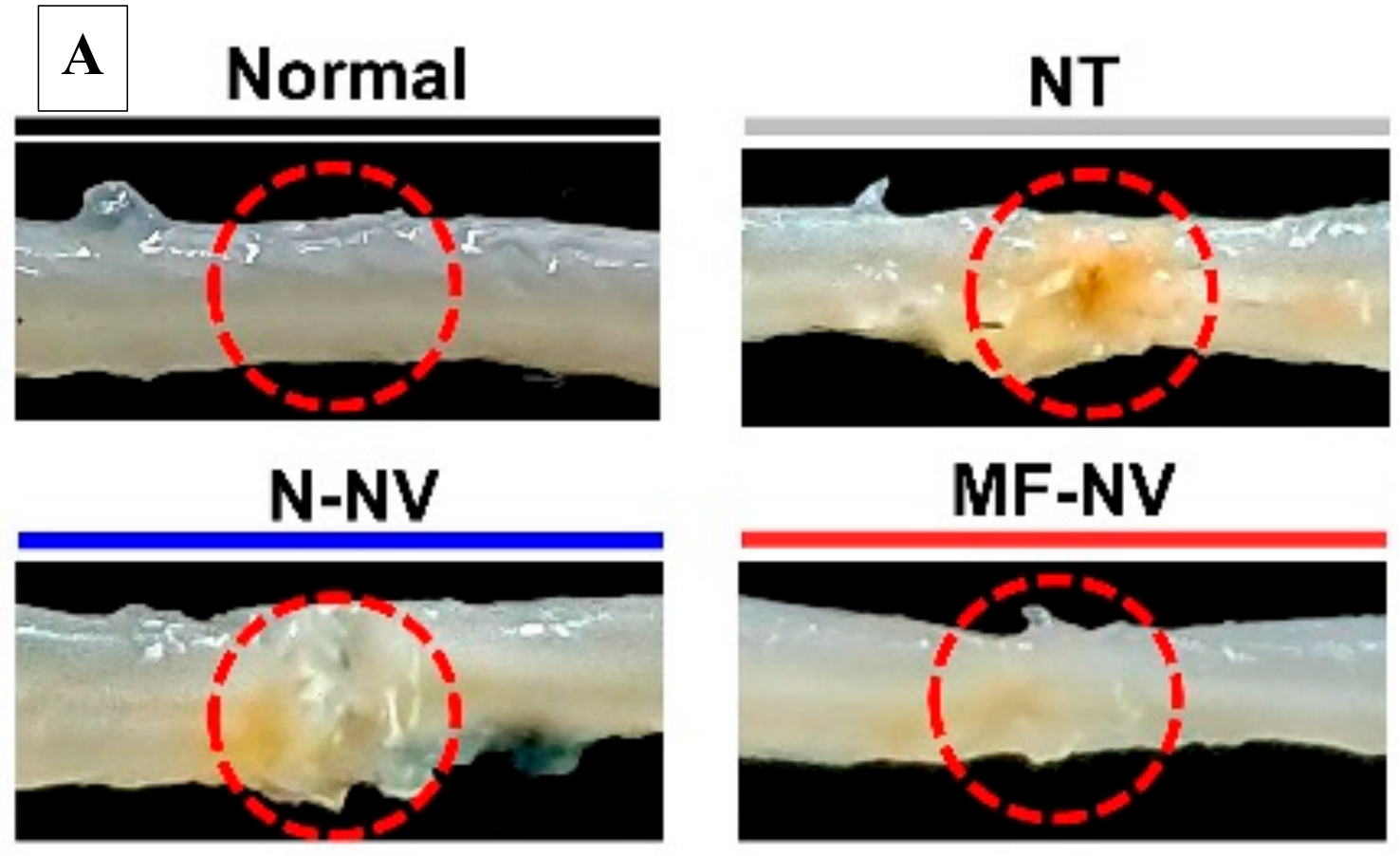


Figure 7: Schematic of the fabrication and therapeutic effects of macrophage membrane-fused exosome-mimetic nanovesicles (MF-NVs). **A**: Preparation of MF-MSCs through the fusion of macrophage (Mf) membranes into MSCs. **B**: Production of MF-NVs from MF-MSCs by serial extrusion. **C**: Intravenous injection of MF-NVs into the spinal cord injured mouse. **D**: Mechanisms of targeting and therapeutic effects of MF-NVs. (ref #15 open access)



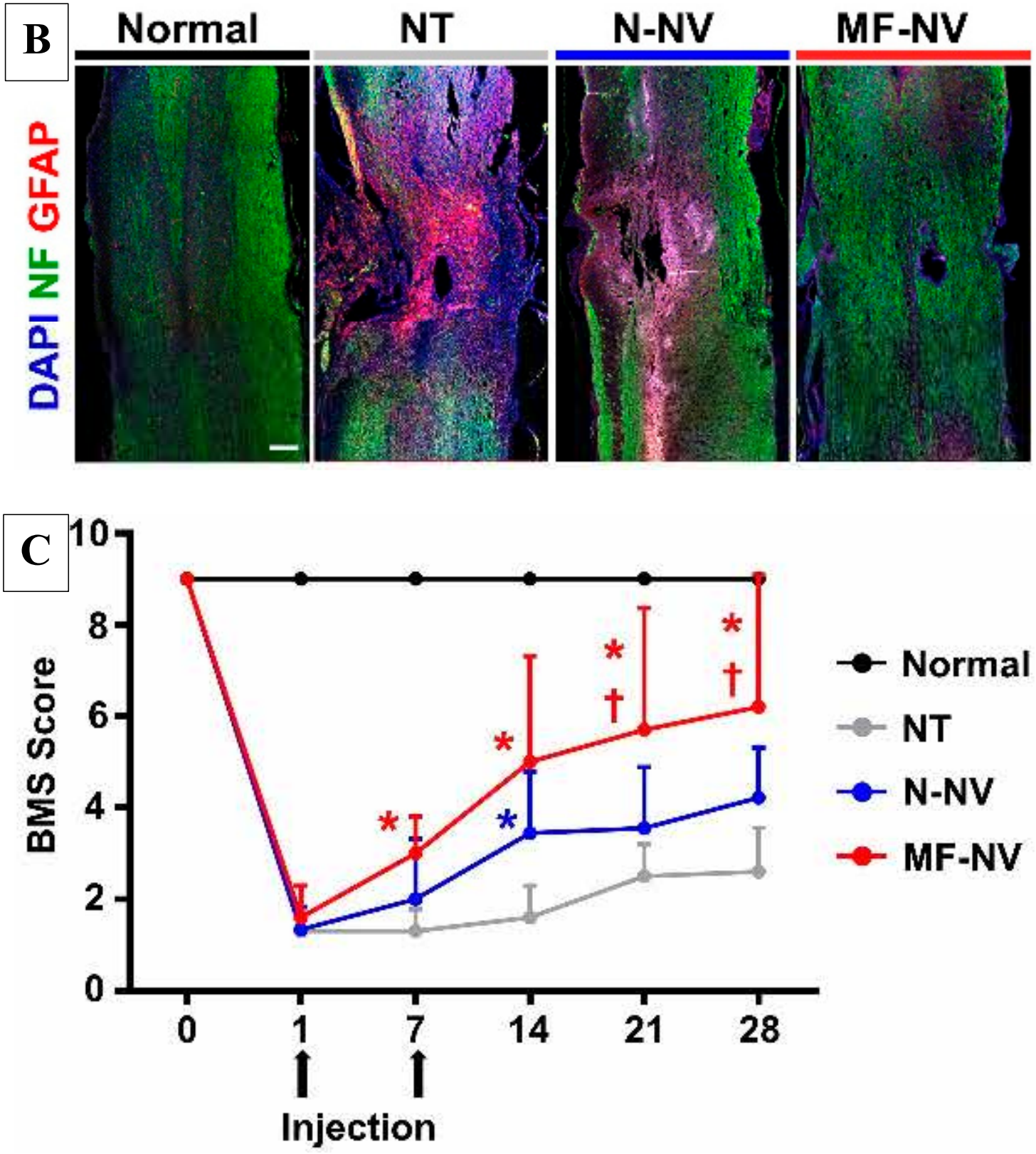


Figure 8: A: Images of spinal cord retrieved 28 days after the NV injection. Dotted red circles indicate the lesion core. **B**: Immunohistochemical staining in longitudinal sections of spinal cord 28 days post-injury (4 animals per group). Scale bars, 20 mm. Neuron (neurofilament (NF), green); astrogliosis (glial fibrillary acidic protein (GFAP), red); DNA (4′,6-diamidino-2-phenylindole (DAPI), purple). **C**: Basso mouse scale (BMS) 28 days post-injury (8 animals pre group). * $p < 0.05$ versus NT and † $p < 0.05$ versus N-NV by using two-way ANOVA followed by post-hoc Bonferroni test. All values are mean ± SD. NT indicates no treatment. (ref #15 open access)

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