Controllable and automated system for synthesizing GMP magnetic nanoparticles for regenerative medicine applications

Dan Dragomir-Daescu, Ph.D.[1], Brandon J. Tefft, Ph.D.[2], Kent D. Carlson, Ph.D.[1],J. Jonathan Harburn, Ph.D.[3], Manju Kalra, M.B.B.S.[4], Amir Lerman, M.D.[2], Gurpreet S. Sandhu, M.D., Ph.D.[2],
[1]Department of Physiology and Biomedical Engineering, [2]Department of Cardiovascular Diseases, [4]Department of Vascular Surgery, **Mayo Clinic, Rochester, MN**
[3]School of Medicine, Pharmacy and Health, **Durham University, Durham, England, UK**

RMM Project Summary

- Goal: Purchase, implement and develop standard operating procedures for research laboratory equipment (Fig. 1) that can be used to synthesize Good Manufacturing Practice (GMP) magnetic nanoparticles
- These nanoparticles can be utilized in a variety of regenerative medicine applications including targeted delivery of cells, growth factors, drugs, genes, and other therapeutic agents
- Creating high-quality nanoparticles using GMP will be critical for translating this promising regenerative medicine strategy into clinical practice, as GMP is an FDA requirement
- With new system, nanoparticle production yield will increase to the order of grams per day (1000x improvement over our current process)
- Increased capacity will allow us to expand our regenerative medicine research efforts, as well as providing a shared resource for other investigators
- System also allows for ready scale-up in the future, which will be critical for delivering these promising therapeutic strategies to patients in Minnesota and worldwide

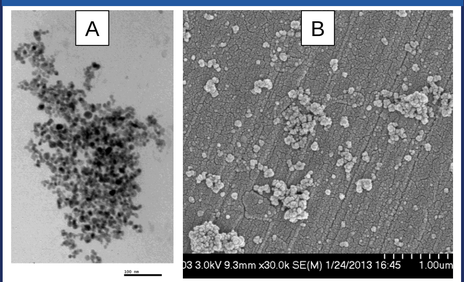
Figure 1

Photographs of Syrris products to be purchased for nanoparticle synthesis: (A) Atlas HD batch system, and (B) Asia flow system.

Background

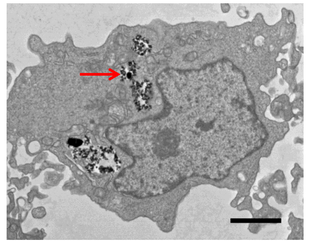
- Our group developed magnetic nanoparticles using the non-hydrolytic thermal decomposition method
- This results in 10 nm magnetite cores within a 50 nm thick shell of poly(lactic-co-glycolic) acid (PLGA) (Fig. 2).
- We have shown that these particles can be used to safely label endothelial cells (Fig. 3) and direct their motion through an aqueous medium using magnetic forces

Figure 2



The left transmission electron micrograph (A) shows the magnetite cores. These are approximately 10 nm in diameter. The right scanning electron micrograph (B) shows the final magnetic nanoparticles. These are approximately 110 nm in diameter.

Figure 3

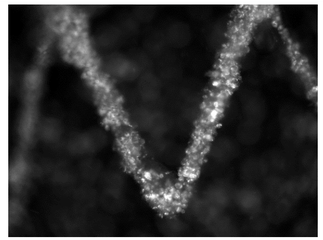


Transmission electron micrograph showing an endothelial cell labeled with magnetic nanoparticles. The magnetic nanoparticles appear as dark black circles clustered together in cytoplasmic endosomes (as indicated by red arrow). Scale bar = 2 µm.

Example Application

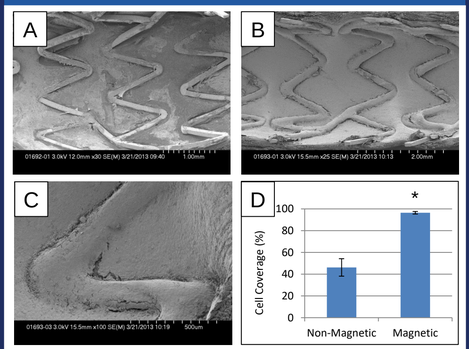
- Our group used magnetic nanoparticles to label endothelial cells and capture these cells to a magnetic stent (Fig. 4)
- Magnetic stents showed significantly improved endothelial cell coverage at day 3 in a large-animal model (96.4 ± 1.1%) when compared to non-magnetic stents (46.1 ± 8.0%) (Fig. 5)

Figure 4



Fluorescence micrograph of a magnetic stent showing capture of fluorescently and magnetically labeled endothelial cells (white).

Figure 5

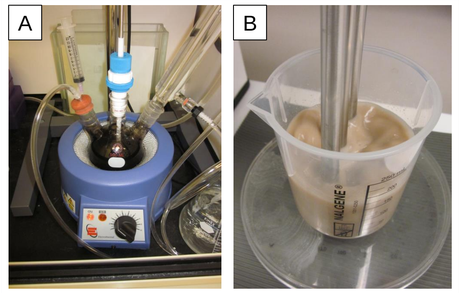


Scanning electron micrographs of vessels treated with (A) non-magnetic and (B,C) magnetic stents at 3 days following implantation in a pig. These images were used to measure the (D) percentage of stent strut area covered by cells as a measure of healing. * $p < 0.05$.

Process Improvement

- Our current method of synthesizing magnetic nanoparticles is time consuming, operator-dependent, and inconsistent (Fig. 6)
- A yield of 40 mg typically takes 2-3 weeks
- The yield, purity, and particle characteristics can fluctuate from batch to batch, even from the same operator
- Syrris Atlas HD-Asia system (Fig. 1) is an automated and controllable GMP-compliant system for nanoparticle synthesis
  - Atlas HD is a batch chemistry reactor that can be used for synthesis of magnetite nanoparticle cores
  - Asia is a flow chemistry reactor that can be used to PLGA coat magnetite cores
  - Syrris system is software-controlled (temperature, pH, stir speed, flow rate, residence time)
- System will allow us to quickly, efficiently and consistently synthesize safe and effective nanoparticles
- Magnetic nanoparticle yield will increase to grams per day

Figure 6



The thermal decomposition method (A) for synthesizing magnetite nanoparticles. High-speed emulsification method (B) for coating the magnetite nanoparticles with PLGA. Following these processes, several weeks of washing cycles are needed to achieve reasonable purity; however, this results in loss of particles and significantly lowers the final yield.

Regenerative Medicine Impact

- Addition of the Syrris system to the Mayo Clinic Center for Regenerative Medicine's Biomaterials and Biomolecules GMP Facility will accelerate the development of magnetic and nonmagnetic nanoparticle-based therapeutics for regenerative medicine applications
- Since FDA approval requires conformity with GMP standards, implementation of the proposed system is necessary to deliver to patients the regenerative therapies developed with the nanoparticles the system creates
- This Syrris system and the standard operating procedures we will develop will enable this shared resource to serve as a platform for novel regenerative medicine research projects

Potential Applications

Regenerative Medicine

- Coat magnetic stents, stent-grafts, and grafts with endothelial cells to improve their blood compatibility
- Target cells to cardiac valves and acellular xenogeneic scaffolds
- Direct stem cells, growth factors, drugs, and other therapeutic agents to target sites within the body

Other Applications

- Targeted delivery of oncolytic agents to tumors
- PLGA nanoparticles for enhanced ultrasound contrast

Acknowledgements

This project is sponsored by a Biobusiness/ Biotechnology grant from Regenerative Medicine Minnesota (Award #RMM-2017-BB-02).