FINAL DRAFT FOR BIOMED 101

GENETICALLY MAGNETIZED PLGA NANOPARTICLES FOR INTRAVENOUS DRUG AND GENE TARGETING

by

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GENETICALLY MAGNETIZED PLGA NANOPARTICLES FOR INTRAVENOUS DRUG AND GENE TARGETING

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New drug targeting system is proposed by incorporating PLGA – poly(D,L-lactide-*co*-glycolide) – nanoparticles and genetically synthesized magnetic nanoparticles. By taking the advantages of PLGA (good biocompatibility, biodegradability, and small size for intravenous delivery) and magnetite (rapid and easy treatments), a powerful drug/gene carrier is made. Recently, many people worked about the synthesis of PLGA nanoparticles for drug delivery (not targeting), and the synthesis of magnetic nanoparticles as a contrast agent for MRI (magnetic resonance imaging). However, there are no published reports for magnetic targeting with PLGA nanoparticles. The main problem is 'how we can incorporate magnetite and biocompatible carrier (PLGA) together.' We will use the lipid-layered magnetic nanoparticles made by microorganisms, with recombinant DNA technology. This lipid layer gives biocompatibility to the magnetic particles and this compatibility enables to be easily incorporated with PLGA. The main objectives are (1) synthesis of genetically magnetized PLGA nanoparticles, and (2) their application to the drug and gene (DNA) targeting.

Keywords: drug targeting, gene targeting, gene therapy, PLGA, magnetite, nanoparticles, recombinant DNA technology.

I. IDENTIFICATION AND SIGNIFICANCE OF THE PROBLEM

Nowadays, drug delivery (including both controlled release and drug targeting) becomes more popular both in academia and industry than ever. The main foci are now shifting to gene delivery for therapeutic purposes and the guided targeting to specific organs. An extremely small-sized carrier is also required for the intravenous injection to prevent the carrier's clogging up in vessels – the size below 70 nm is generally demanded, primarily due to the size constraints within the capillary vessels. The carrier should also be biodegradable and biocompatible. PLGA – poly(D,L-lactide-*co*-glycolide) – nanoparticles, with PVAL – poly(vinyl alcohol) – as a stabilizer, are generally considered as an acceptable carrier to meet the above all requirements. Many problems still remain such as instability, long-term incompatibility with blood cells and tissues, and non-zero-order release behaviors.

Targeting methodology is another problem. One of the most favored methods for guided targeting is a magnetic one, especially with magnetite (Fe_3O_4). However, conventional magnetite particles are so big that they cannot be incorporated within the nanoparticles. A recently introduced alternative is ferrofluid, but no work has been published that incorporates PLGA nanoparticles and the ferrofluid. The main problem is the incompatibility between PLGA and the ferrofluid. The advantages of magnetic targeting – rapidity and easy treatment – are too beneficial to be abandoned.

Therefore, for the magnetic targeting of drugs (or genes), we must choose one method from the followings: (Note: particle size cannot be sacrificed – it must be smaller than 70 nm.)

(1) To use alternative materials (for example, polystyrene-based latex particles) rather than PLGA, that can be easily incorporated with ferrofluid or magnetite. This alternative generally has lower biocompatibility than PLGA.

(2) To adopt alternative targeting method, such as using site-specific enzymes or using targeting by antibodies, rather than magnetic targeting. Targeting efficiency are generally lowered comparing with the magnetic one.

Table 1 summarizes several reviews on the intravenous drug targeting (Guiot and Couvreur, 1986, Rembaum and Tokes, 1988, Tsuruta et al., 1993). PS (polystyrene) and its derivatives were widely used as carriers at first, but people gradually moved to PEU (polyether urethane) and PLGA to increase the biocompatibility. Conversely, these new carriers' incorporation with magnetite is more difficult than PS, hence these carriers are currently used only for controlled release, not targeting.

Targeting methodolgy is also shown in Table 1 (Guiot and Couvreur, 1986, Rembaum and Tokes, 1988, Kompala and Todd, 1991). Magnetic targeting is superior to other methods in all aspects (except in site-specific efficiency), but making biocompatible nano-sized one remains as a big problem. Therefore, the use of magnetic particles is confined to *ex vivo*, except the use as an MRI (magnetic resonance imaging) contrast agent.

Table 1. Current status in intravenous drug targeting.

Carrier selection	Biocompatibility	Incorporation with magnetite
PS and its derivatives	Poor	Good
PEU and its derivatives	Not bad	Not bad
PLGA and its derivatives	Good	Poor
Proteins (albumin, dextran)	Good	Not tested yet

Targeting methodology	Speed	Treatment	Site-specific
Magnetic	Fastest	Easy	Good
Enzymatic	Rather slow	Complex	Arguable
Immunological	Fast	Complex	Excellent

Note. 'Carrier selection' information is based on the results of controlled release system, not targeting or site-specific system.

II. BACKGROUND, TECHNICAL APPROACH, AND ANTICIPATED BENEFITS

II.1. Background

Previous Applications Using Magnetic Beads

Magnetite (Fe_3O_4) has been widely used in many organic and inorganic chemical applications due to its ease of treatment, but it is recently highlighted in the biomedical area. Magnetite particles can be easily and rapidly separated by an externally applied magnetic field. Due to the ease and rapidity of response, these particles are alternatives for biomedical applications. Cell separation and MRI (magnetic resonance imaging) were the first areas that magnetite was used in biomedical applications.

(1) Cell Separation – Many different kinds of cells are tested in clinical labs, and these cells must be separated for specific purpose. Two methods are commonly used (Kompala and Todd, 1999) – flow cytometry and immunomagnetic cell separation (IMCS). Although there exists a state-of-the-art flow cytometry, IMCS is commonly used in most labs, because IMCS is faster and easier to handle than flow cytometry. In IMCS, polymer beads containing magnetite are attached with monoclonal antibodies, which can be specifically bound to target cells. An external magnetic field keeps these beads stationary (Schwarz and Contescu, 1999); so the target cells are bound to the flow system and other cells flows through. For polymer beads, magnetized polystyrene (PS) is the most common one used (these beads are commercially available). Magnetite does not appear to sustain permanent magnetization; hence magnetic interaction between particles is negligible in aqueous media (Sohn et al., 1996). However, in our opinion, there probably exist a stability problem, because these magnetized beads have relatively high PDI (polydispersity index) values, which means the size distribution is broad (Ding et al., 1998). This mainly results from the polymerization methods – dispersion polymerization with solvent evaporation technique – which necessarily leads to broad size distribution. In addition, for the same reason, these particles are so big that they cannot be used for intravenous injection. A recent study by Partington et al. (1999) concerns the synthesis of magnetic nanoparticles

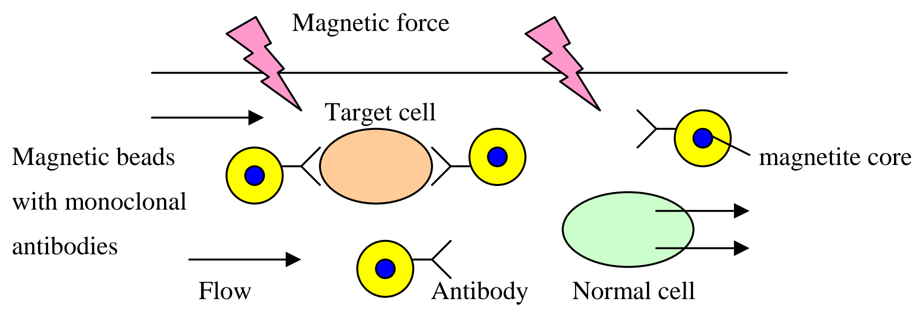

Figure 1. Immunomagnetic Cell Separation

for use with cell separation. However, there still exists a problem that they cannot be incorporated with polymers, which can be used to carry drugs or genes (Schwarz and Contescu, 1999).

(2) MRI (magnetic resonance imaging) – magnetite particles have been used as a magnetic resonance contrast agents. These particles are small enough to prevent the clogging problems, but this application is still plagued by problems in incorporating them with polymers. Recently, Zaitsev et al. (1999) published a paper about the optical properties of polymer-coated magnetic nanoparticles, but made no attempts to use it as a drug/gene carrier. Biocompatibility with human tissues/organs and drugs/genes continues to be a persistent problem for these particles.

(3) Other applications: magnetite particles have also been used as an immobilization matrix (Bahar and Celebi, 1998), and also as a solid-phase matrix of ELISA (enzyme linked immunosorbent assay) (Kala et al., 1997).

PLGA Nanoparticles as a Drug Carrier

Nanoparticles are one of the most promising dosage forms of potential formulations for site-specific drug delivery systems including drug targeting. Special interest has been focused on the particles prepared from polyesters, such as poly(D,L-lactide-*co*-glycolide) (PLGA), poly(D,L-lactide) (PLA), polyglycolide (PGA). This is largely due to their biocompatibility and resorbability via natural pathways. Moreover, the Food and Drug Administration (FDA) has approved the use of polymers prepared from glycolic acid and lactic acid for *in vivo* use. These polymers do not need surgical removal after the completion of drug dosage.

These nanoparticles were made by the following methods:

(1) Emulsion-evaporation method (in 1980's): This method utilizes the o/w (oil-in-water) emulsion. Oil-phase solvent is removed by evaporation, thus forming particles. From this reason, this method is also called as 'Solvent Evaporation Method.' The size of resulting particles are generally over 1 μm, thus nanoparticle formation is difficult with this method (Guiot and Couvreur, 1986, Ibrahim et al., 1991).

(2) Emulsion-diffusion method (in 1990's): This method was evolved from the 'salting-out' procedure (Allemann et al., 1992, 1993). The difference between emulsion-evaporation and emulsion-diffusion methods is that the particle formation is induced by diffusion, not evaporation. Nanoparticle formation is possible in this method, with the help of stabilizers. At first, benzyl alcohol was used as an oil-phase solvent, and poly(vinyl alcohol) (PVAL) was used as stabilizers (Leroux et al., 1995). Quintanar-Guerrero et al. (1998) used propylene carbonate (PC) as a solvent instead of benzyl alcohol, to reduce the toxicity of oil-phase solvent. Labhasetwar et al. (1998) also reported that using didodecyldimethylammonioum bromide (DMAB) as a co-stabilizer could enhance the loading level of drugs.

Gene Delivery and Targeting for Gene Therapy

Many diseases are related to defects in genes. Therefore, site-specific gene delivery may be of great promise to those who suffer from diseases with genetic origin. Nowadays, the genes can be synthesized for therapeutic purpose very easily. The mass of genes (DNA's) can be increased through PCR (polymerase chain reaction). (Glick and Pasternak, 1998).

Genes, in cDNA form that has no introns (cDNA is made by reverse transcription from mRNA), are carried by a biocompatible matrix. For site-specific delivery, both enzymatic and magnetic methods can be used. Genes can be attached to the surface of carrier beads, or they can be encapsulated into porous nanocapsules that mimic the eukaryotic nuclei (Figure 2).

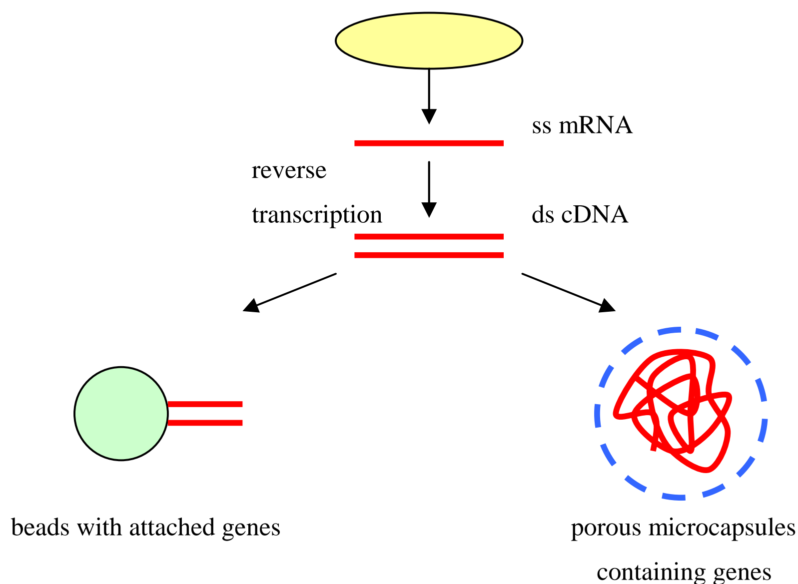

Figure 2. Two methods for site-specific gene targeting

Biocompatible Magnetic Particles by Microorganisms

Matsunaga et al. (1998) reported that certain types of microorganisms are capable of producing biocompatible magnetic nanoparticles. The microorganism is *Magnetospirillum* sp. AMB-1, and it synthesizes intracellular particles of magnetite (Fe_3O_4). With the help of recombinant DNA technology, the gene for this magnetite production (*macA* gene) was cut by restriction enzymes (*Bam*HI, *Sac*I), and ligated to plasmid vector pRK415.

They only suggested its use with immunoassays, but it has the potential for magnetic drug/gene targeting as well. This magnetite has high biocompatiblity, due to the biologic manufacture, especially the lipid layer on its surface. This biocompatibility is important for PLGA incorporation.

II.2. Technical Approach and Anticipated Benefits

Our approach for new method of magnetic drug/gene targeting is as follows:

Step 1. Production of magnetic particles by recombinant DNA technique

With this technique, we can avoid the incompatibility problem between conventional magnetite or ferrofluid and PLGA. This procedure is schematically illustrated in Figure 3.

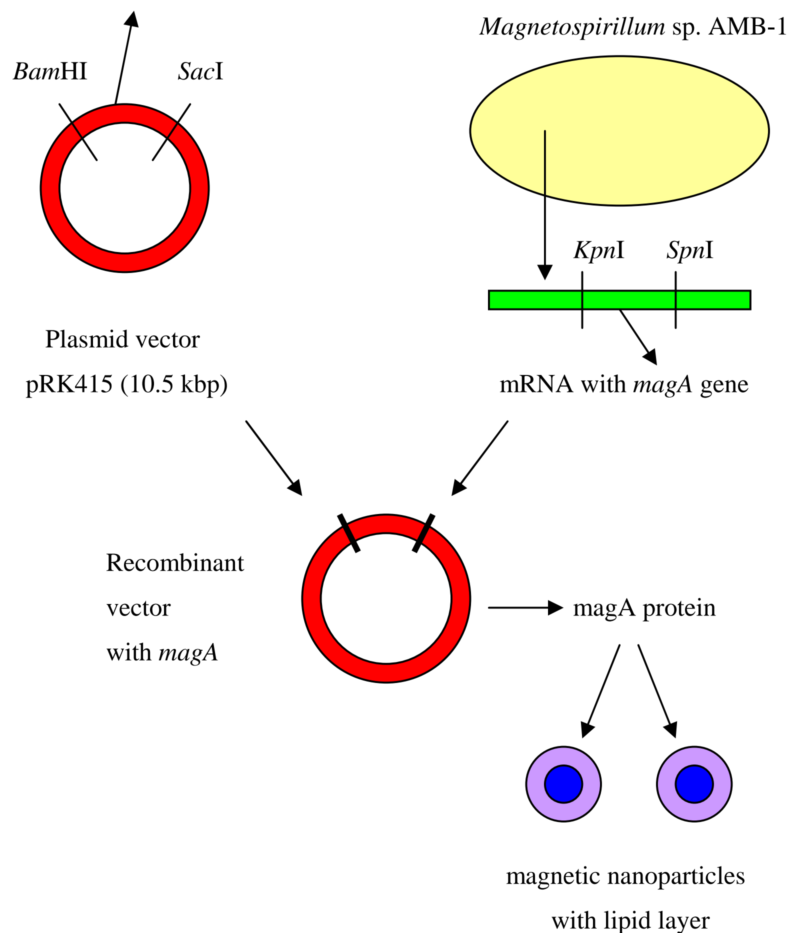

Figure 3. Biocompatible magnetic nanoparticle formation

(1) Preparation of plasmid vector template: a plasmid vector, pRK415 will be used. Its size is 10.5 kbp. Two restriction enzymes, *Bam*HI and *Sac*I will cut this vector to make a template for *magA* gene expression.

(2) Preparation of *magA* gene: mRNA (which has no introns) containing *magA* gene will be separated from *Magnetospirillum* sp. AMB-1. Two restriction enzymes, *Kpn*I and *Spn*I will digest this mRNA to obtain *magA* gene.
(3) Preparation of recombinant vector: *magA* gene will be ligated to the plasmid vector template. This recombinant vector will be inserted in *E. coli* to express magA protein.
(4) Production of lipid-layered nanoparticles: magA protein will produce lipid-layerd, biocompatible magnetic nanoparticles (BMNP).

Step 2. Incorporation the biocompatible magentic nanoparticles (BMNP) with PLGA

The resulting magnetic particles have lipid layer, which provides intrinsic biocompatibility. Adding PLGA will yield the core-shell structure. The following is a summary for making the structured biocompatible magnetized PLGA nanoparticles. Figure 4 also illustrates this procedure.

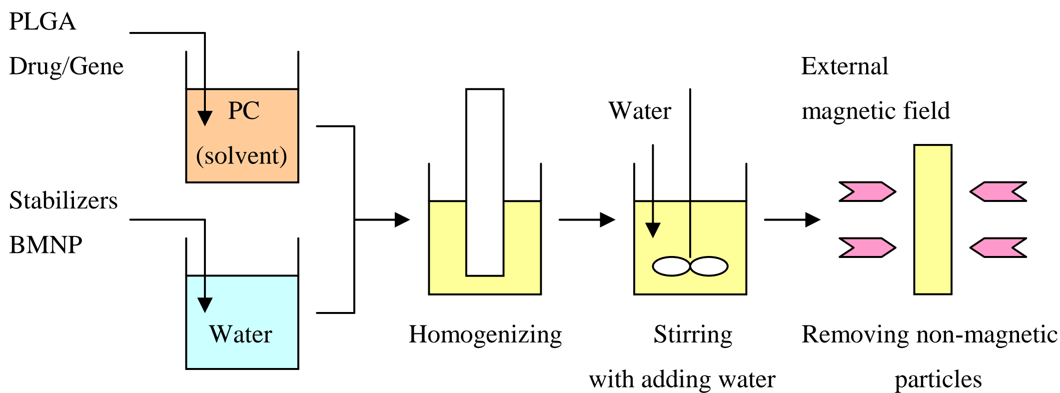

Figure 4. Procedure for biocompatible magnetized PLGA nanoparticle production

(1) Preparation of organic (oil) phase: PLGA (and drugs/genes) are dissolved in organic solvent, PC (after Quintanar-Guerrero et al., 1998). PLGA can be fluorescent-labeled with rhodamine B, for tissue localization studies.
(2) Preparation of aqueous phase: Stabilizers, PVAL and DMAB (after Labhasetwar et al., 1998) are dissolved in water. We will also dissolve BMNP in aqueous phase; thus micelles with BMNP can be generated at this stage. BMNP disperse very well in aqueous phase because they are covered with a stable lipid layer (Matsunaga and Takeyama, 1998).
(3) Emulsification: Mixing and homogenization of the above two phases yield an o/w emulsion. High-speed homogenizer required in this step.

(4) Nanoparticle formation: Water is subsequently added to the o/w emulsion, leading to the diffusion of PLGA (and drugs/genes) to the micelles in aqueous phase. This yields the core-shell structured nanoparticle formation. Figure 5 schematically illustrates the nanoparticle formation.

(5) Removal of non-magnetic nanoparticles: There probably exist non-magnetized nanoparticles, that is, PLGA nanoparticles without BMNP. These non-specific nanoparticles can be easily removed by an external permanent magnet (Schwarz and Contescu, 1999).

(6) Purification: Core-shell structured nanoparticles are purified by dialysis. Freeze-drying produces a homogeneous free-flowing powder.

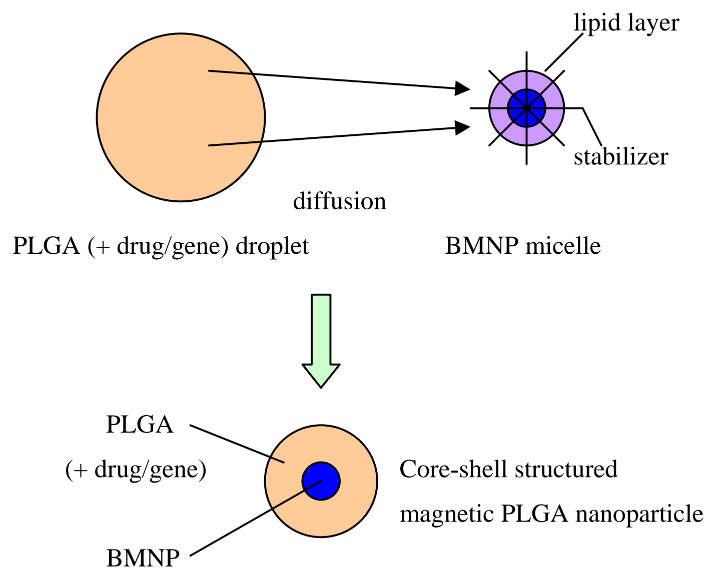

Figure 5. Nanoparticle formation

Step 3. In vitro *study (for phase II)*

Some feasibility tests should be performed *in vitro* prior to *in vivo* experiments. Following is a list of factors that could determine the PLGA-BMNP feasibility for biomedical use.

(1) Stability: The particles should have enough stability to overcome non-Newtonian behavior *in vivo* conditions. This can be measured through HLB (hydrophile-lipophile balance), CMC (critical micelle concentration), and hydrophobicity values (Shaw 1992). Surface tension meter can calculate HLB and CMC values. Hydrophobicity can be measured by labeling pyrene (strongly hydrophobic material) to the stabilizers.

(2) Particle size distribution: Nanoparticles should also be uniform in size to prevent an aggregation. Dynamic light scattering (DLS) should be used for size determination because the PLGA-BMNP's are smaller than 0.1 μm.

(3) Drug/gene content: The particles should have sufficient loading amount of drugs/genes. Centrifuging and spectroscopy techniques will be adopted.

(4) Movement of particles in a magnetic field: The particles should behave properly in blood vessels where magnetic field is applied. Clogging with the vessels, self-aggregation, and decomposition are not allowed for nanoparticles. Mathematical simulation and experimental certification will be simultaneously performed. The magnetic force will be applied as a function of flat, sine-wave, or impulse (Figure 6).

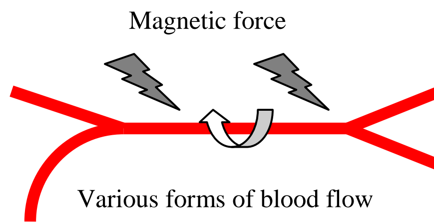

Figure 6. Motion of nanoparticles in blood vessels

Step 4. In vivo *study (for phase III)*

In vivo experiments for animal will be performed. Localization, retention, and bioavailability of the nanoparticles will be evaluated. Fluorescent-labeled (rhodamine B) nanoparticles will be used to determine the tissue localization and persistence of them of them in the tissue.

Injury will be induced in rats, then PLGA-BMNP will be infused to them. External permanent magnets will be attached to the skin (just above the specific tissue or organ) of rats. The rats will be killed with varying the time (such as 1 day, 7 days, and 1 month), then the above parameters – localization, retention, and bioavailability will be measured.

III. PHASE I TECHNICAL OBJECTIVES

The long-term goal of this program is to demonstrate the targeted drug delivery using magnetized particles *in vivo*. Good biocompatibility of carriers, good stability of magnetized-carriers, as well as rapidity and ease of magnetic targeting, is required.

Detail objectives of phase I are listed in the following:

1. To produce magnetized nanoparticles with recombinant DNA technology
   The optimal conditions for the production of biocompatible magnetic nanoparticles (BMNP) should be established; the concentrations of restriction enzymes, the separation of non-specific recombinant vectors, and the culture conditions for *magA* gene expression in *E. coli*. Size distribution of BMNP should also be determined at this stage.
2. To encapsulate these particles in PLGA
   The above BMNP will be encapsulated in PLGA (plus drugs/genes) by emulsion-diffusion method. The optimal experimental conditions should be established; homogenizer speed, stirring rpm, stabilizer concentration, and the rate of water addition.

The followings are the technical objectives of phases II and III.

3. To demonstrate the stability and sufficient drug loading (phase II)
   Various parameters (HLB, CMC, hydrophobicity, and drug/gene content) will be obtained to secure the particle stability and the sufficient drug loading.
4. To demonstrate ability to control the particles in a magnetic field (phase II)
   Mathematical modeling and experimental certification will be carried out to prevent the clogging, self-aggregation, and decomposition of nanoparticles.
5. *In vivo* study (phase III)
   In vivo experiments for animal will be performed to evaluate localization, retention, and bioavailability of the nanoparticles.

IV. PHASE I RESEARCH PLAN

Task 1. Gene Expression with Recombinant DNA Techniques

The objective of this task is to make biocompatible magnetic nanoparticles (BMNP) that can be controlled by magnetic fields. The overall procedure will follow after Matsunaga et al. (1998). Details are listed in the following:

1. Preparation of plasmid vector template
   *Bam*HI and *Sac*I (restriction enzymes) will digest plasmid vector pRK415 (10.5 kbp) to make a template for *magA* insertion. A gel electrophoresis will determine the efficiency of restriction enzymes. Digestive conditions will be changed after this analysis.
2. Preparation of *magA* gene
   The mRNA containing *magA* gene will be separated from *Magnetospirillum* sp. AMB-1 by a gel electrophoresis. Two restriction enzymes, *Kpn*I and *Spn*I, will digest this mRNA to obtain *macA* gene. After sequencing of this gene, PCR technique will be used to amplify the *macA* gene. (Two primers are required for PCR.) Gene sequencing and PCR apparatuses are required for this step. For PCR, Primers and dNTP's are required in excess, and *Taq* DNA polymerase is required for the stability in high temperature.
3. Preparation of recombinant vector
   With the help of blunt-end ligase (the above restriction enzymes makes a blunt-end), *magA* gene will be ligated to plasmid vector template. This recombinant vector will be inserted in *E. coli* to obtain magA protein. Batch fermentation is required for the gene expression in *E. coli*.
4. Production of BMNP
   magA protein will make lipid-layerd BMNP. BMNP will be separated from cell cultures by centrifugation. Optimal rpm should be determined to prevent the breaking-up of BMNP. Further purification will be made by an external magnetic field, shown in the following figure.

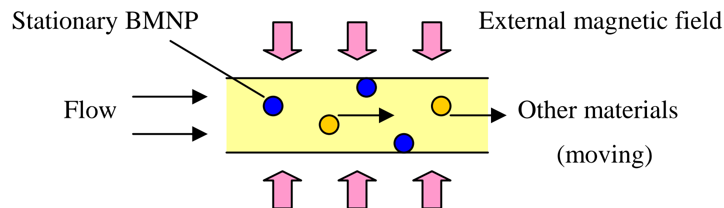

Figure 7. Purification of BMNP from cell culture

5. Characterization of BMNP

   The size distribution will be determined by DLS (dynamic light scattering). Raman spectroscopy, conductometric titration, and zeta potential analysis will give additional information of the surface properties. A recent study by Vo-Dinh et al. (1999) reports that the surface-enhanced Raman scattering (SERS) can be used for analysis of biological compounds in multicomponent environment. Therefore, we will use SERS technique to determine the lipid content in BMNP. Conductometric titration and zeta potential analysis gives direct information of electrostatic stability (Yoon et al., 1996, 1998b, 1999). The stability will be measured by HLB and CMC values, through surface tension meter.

The overall scheme was already illustrated in Figure 3. The following table summarizes the materials and apparatus required for task 1.

Table 2. Materials and Apparatus Required for Task 1

Step	Materials	Apparatus required
Step 1 – plasmid vector template	pRK415	Gel electrophoresis
	*Bam*HI, *Sac*I	
Step 2 – *magA* gene	*Magnetospirillum* sp. AMB-1	Gel electrophoresis
	*Kpn*I, *Spn*I	Gene sequencing apparatus
	Primers	PCR apparatus
	dNTP	
	Taq DNA polymerase	
Step 3 – recombinant vector	Step 1 and 2 products	Fermenter
	Blunt-end ligase	
	E. coli, cell culture	
Step 4 – BMNP	Step 3 product	Centrifuge
		Column with magnetic field
Step 5 – characterization	Step 4 product	DLS
		SERS
		Conductometric titrator
		Zeta potential analyzer
		Surface tension meter

Task 2. Magnetized PLGA nanoparticle formation

In this task, PLGA and BMNP will be incorporated into one, resulting core-shell structure (core: magnetic beads, shell: PLGA). The overall scheme was already illustrated in Figure 4, and details are listed in the following:

1. Preparation of organic (oil) phase
   PLGA (plus drugs or genes) will be dissolved in organic solvent, PC, after Quintanar-Guerrero et al. (1998).
2. Preparation of aqueous phase
   Stabilizers, PVAL and DMAB will be dissolved in water, after Labhasetwar et al. (1998). Then BMNP will be added to this aqueous phase, thus forming micelle with BMNP core. (Figure 5). The amount of PVAL will determine the final size and stability of PLGA-BMNP, and that of DMAB will determine the drug/gene content.
3. Emulsification
   The above two phases will be mixed together and homogenized at high speed. Mixing ratio and homogenizer speed will be varied. The o/w emulsion will be made at this step. PLGA (plus drugs/genes) exist in organic (oil) phase, and BMNP in aqueous (water) phase.
4. Nanoparticle formation
   Water will be subsequently added to the o/w emulsion, leading to the diffusion of PLGA (plus drugs/genes) to the micelles in aqueous phase. This yields the core-shell structured nanoparticle formation, and it was illustrated in Figure 5. The only variable in this step is the rate of water addition.
5. Removal of non-magnetic nanoparticles
   The non-specific nanoparticles (which has no BMNP in core) will be removed by the same method illustrated in Figure 7.
6. Purification
   Resulting magnetized PLGA nanoparticles will be purified by dialysis and freeze-drying.
7. Characterization
   The characterization of magnetized PLGA nanoparticles will be performed by a similar method of task 1. Size distribution will be measured by DLS, surface acidity by conductometric titration, and electrostatic stability by zeta potential analyzer (Yoon et al., 1996, 1998b, 1999). Further characterization studies – HLB, CMC values, hydrophobicity, and drug/gene content – will be performed in phase II.

Table 3 summarizes the materials and apparatus required for task 2.

Table 3. Materials and Apparatus Required for Task 2

Step	Materials	Apparatus required
Step 1 – organic phase	PLGA Drug/Gene PC	
Step 2 – aqueous phase	PVAL, DMAB BMNP Water	
Step 3 – emulsification	Step 1 and 2 products	Homogenizer
Step 4 – nanoparticle formation	Step 3 product (o/w emulsion) Water	Stirrer Micro tubing pump
Step 5 – removal of non-magnetic particles	Step 4 product	Column with magnetic field
Step 6 – purification	Step 5 product	Dialysis Freeze-dryer
Step 7 – characterization	Step 6 product	DLS Conductometric titrator Zeta potential analyzer

V. RELATED RESEARCH AND DEVELOPMENT

Major recent researches (biomedical application of magnetic particles or non-magnetic nanoparticles) are already mentioned in previous sections. These works can be classified into several categories:

1. Microparticles, non-magnetized
   Yoon et al., 1996, 1998b, 1999, for protein adsorption study
   Yoon et al., 1998a, Lee et al., 1998, for protein separation
   - Microparticles generally have the size between 0.1~10 μm. J.-Y. Yoon (who will work as a graduate student researcher in this project) have published several works with the microparticles ranging 0.2~0.4 μm to reveal the interactions between proteins and polymer surfaces. This *a priori* knowledge will be useful for studying drug-PLGA interaction.
2. Microparticles, magnetized
   Bahar and Celebi, 1998, for immoblization of enzymes
   Liberti and Pino, US Patent, for cell separation
   Ding et al., 1998, for cell separation
   - Magnetized microparticles for cell separation generally have large size, ranging 1~100 μm. Especially, cell separation requires large particles due to the size of living cells. These works are intended for *ex vivo* use, and not applicable for *in vivo* use.

   Kala et al., 1997, for immunoassay
   Sohn et al., 1996, for optical study
   - Magnetic microparticles were tested for bio-separation and immunoassay in these works. The size of particles are relatively small (below 1 μm), because they employed a light scattering technique.
3. Nanoparticles, non-magnetized
   Allemann et al., 1993, for controlled release of drugs
   Leroux et al., 1995, establishment of emulsion-diffusion method
   Quintanar-Guerrero et al., 1998, change of solvent to PC to increase biocompatibility
   Labhatsetwar et al., 1998, introduction of DMAB for the increase in drug content
   - All of the above works were related with the synthesis of nano-sized PLA drug carrier. Allemann et al. established a salting-out procedure, and Leroux et al. developed an emulsion-diffusion method to make nanoparticles. Quintanar-Guerrero et al. and Labhatsetwar et al. improved biocompatibility and drug content, respectively.

   Maruyama et al., 1999, for gene delivery
   - A recent study by Maruyama et al. dealt with gene (DNA) delivery, instead of drug. Their primary objective was gene therapy (to treat the disease caused by a gene defect). Non-magnetized nanoparticles were used.

4. Nanoparticles, magnetized

Matsunaga et al., 1998, for MRI

- Biocompatible, magnetized nanoparticles were prepared by the recombinant DNA technology. Their primary concern is to use them as an MRI contrast agent, hence they did not incorporated with any polymer carriers.

Partington et al., 1999, for cell separation

- Large-sized magnetized PS (polystyrene) particles are generally used for cell separation, but these people used nano-sized particles to improve the selectivity. However, they eventually made microparticles, because the nanoparticles were assembled together while passing several steps.

Sonti et al., 1997, for DNA separation

- The authors made a nanocluster from magnetic nanoparticles to separate DNA. These particles lack biocompatibility and stability, hence they are not suitable for *in vivo* use.

Our only concern is group 4, because we need magnetized drug/gene targeting and nano-size for *in vivo* use. No attempts have been made to use magnetized nanoparticles as a drug carrier. While it is possible to make magnetic nanoparticles, there are no published reports dealing with the incorporation of these magnetite nanoparticles and carriers. Partington et al. eventually made microparticles for cell separation (nanoparticles are not suitable for cell separation media, they are so small). The particles that Sonti et al. made are actually not particles; it is nanoclusters. Therefore, only the work by Matsunaga et al. is valuable, although they made without any carriers or drugs and just applied to MRI.

The previous works performed by J.-Y. Yoon (which dealt with the interfacial phenomena between biomolecules and polymeric particle surfaces) will make a basis for Task 2 in Phase I, especially for drug content and releasing behavior.

VI. KEY PERSONNEL AND BIBLIOGRAPHY

Professor Robin L. Garrell (Department of Chemistry and Biochemistry & Biomedical Engineering Program, University of California, Los Angeles) will serve as principal investigator. She will supervise the whole program, and also take charge of the polymer synthesis section. Professor Garrell has over 15 years of experience in organic/inorganic synthesis for biological applications, especially in surface chemistry. Graduate student researcher Jeong-Yeol Yoon (Biomedical Engineering Program, University of California, Los Angeles) will take charge of the characterization section, as well as actual experimental section.

VII. FACILITIES AND EQUIPMENT

Biomedical Engineering Program in University of California, Los Angeles has wide resources ranging from School of Engineering and Applied Sciences, School of Medicine, School of Dentistry, as well as some departments from College of Letters and Science. Followings are the required facilities for this program, and all of these are available upon request.

a) Spectrophotometer: UV/Vis, FT-IR, AA, Raman
b) Chromatography: HPLC, TLC, GPC, GC
c) Electron Microscope: SEM, TEM, AFM
d) Surface characterization: Zeta Potential Analyzer, SERS, Surface Tension Meter, Auto-titrator
e) Size analysis: DLS
f) Gel Electrophoresis Equipment, Gene Sequencing Apparatus, PCR Apparatus
g) Fermenter
h) Dialyzer, Freeze-dryer
i) Homogenizer, Centrifuge, Magnetic Stirrer, Micro Tubing Pump
j) Organic Synthetic Laboratory

VIII. CONSULTANTS

Following people are anticipated to work as consultants for DNA manipulation, sequencing, and recombinant technologies, for *magA* gene expression in task 1.

Professor James C. Liao, Department of Chemical Engineering & Biomedical Engineering Program, University of California, Los Angeles
Professor Imke Schroeder, Department of Microbiology and Molecular Genetics & Biomedical Engineering Program, University of California, Los Angeles

IX. POTENTIAL COMMERCIAL APPLICATIONS

Although the method of site-specific magnetic targeting is primitive comparing with the enzymatic and immunological targeting, the ease of treatment and the rapidity are still attractive. Numerous studies were published for the biocompatible magnetic nanoparticles, and for the biocompatible drug carriers. However, incorporating these two materials into one still remains a great problem. If this problem is solved (with the method we suggested in this proposal), it will be applied to the following commercial applications.

(1) Site-specific drug targeting

Drugs are encapsulated into PLGA (shell side of our proposed new carrier), and they are injected into the blood vessels. Drugs are localized on the specific tissue/organ with the help of an external paramagnet (attached to the skin of patient) or an internal paramagnet (inserted into the specific tissue/organ by a surgeon). Internal paramagnet should be treated with biocompatible materials to prevent graft-vs-host disease.

(2) Site-specific gene targeting for gene therapy

Almost all diseases are originated from the defects of genetic codon. Therefore, inserting a 'right' gene into the patients' body can treat the disease. However, this external gene occasionally causes problems due to the excess amount of proteins produced. Site-specific targeting technique can fix this problem, and our new carrier is anticipated to improve the efficiency of localization as well as the cost. The technical aspects are the same as drug targeting.

X. CURRENT AND PENDING SUPPORT

No work substantially similar to that proposed here is being conducted at this time, nor is any pending.

XI. EQUIVALENT PROPOSALS

No proposal substantially similar to this has been submitted to any other agency.

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