

Polymers as a Platform for Drug Delivery: Reviewing our Current Portfolio on Poly(lactide-co-glycolide) (PLGA) Microspheres

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Abstract: The chemical conjugation or the physical embodiment of therapeutic agents in polymers offers great potential to improve the efficacy and safety of therapies and create novel therapeutic opportunities. This has led to numerous concepts regarding the formulation, the delivery and the targeting of therapeutics. Our current research portfolio in this field includes several projects. In all of them poly(lactide-co-glycolide), PLGA, varying in molecular weight and end-group modification, is used as a delivery platform. In this review we will firstly outline our concept of single-injection vaccines. Instead of the application of repeated booster doses, such vaccines are designed to deliver the antigen in a pulsed or sustained fashion, in such a way that protective humoral and cellular immunity can be achieved after a single injection. In the second polymer-based project we aim at the administration of insulin-like growth factor I (IGF-I), which bears great potential in tissue engineering, e.g. to enhance bone healing. Here we summarize on a PLGA microsphere system for the localized and controlled delivery of IGF-I in order to close bone defects after fracture, pathology or surgery. In a third project we use this polymeric platform for the design of antigen-encoding DNA vaccines designed to preferentially target the most potent antigen presenting cells, i.e. the dendritic cells. Two prime objectives are currently being studied, i) enhancement of phagocytosis of DNA-loaded PLGA microspheres by human-derived dendritic cells, and ii) release of intact plasmid DNA and concomitant transfection of dendritic cells once the PLGA microspheres were successfully phagocytosed.

Keywords: Antigen delivery · Biodegradable polymers · Genetic engineering · Morphogen delivery · Poly(lactide-co-glycolide) · Polymeric delivery systems

1. Introduction

The chemical conjugation or the physical embodiment of therapeutic agents in polymers offers great potential to improve the efficacy and safety of therapies and create novel therapeutic opportunities. This has led to numerous concepts regarding the formulation, the delivery and the targeting of therapeutic agents. There are three general mechanisms by which drugs may be delivered from polymeric

systems in a controlled fashion: i) diffusion of the drug species from or through the system, ii) chemical or enzymatic reactions, leading to a degradation of the system or cleavage of the drug from the system, and iii) activation of the system by the environment, e.g. by osmosis or swelling, leading to the controlled release of the embodied agent. A general review on the principles of polymers for drug delivery was previously presented by Langer [1].

Our current research portfolio in this field includes several projects. In all of them poly(lactide-co-glycolide), PLGA, varying in molecular weight and end group modification, is used as a delivery platform. In this review we will firstly outline our concept of single-injection vaccines. Instead of the application of repeated booster doses, such vaccines are designed to deliver the antigen in a pulsed or sustained fashion, in such a

way that protective humoral and cellular immunity can be achieved after a single injection. In the second polymer-based project we aim at the localized administration of insulin-like growth factor, IGF-I, which bears great potential in tissue engineering, e.g. to enhance bone healing. Here we report on a PLGA microsphere system for the controlled localized delivery of IGF-I in bone defects. In a third project we use the same platform for the design of antigen-encoding DNA vaccines designed to preferentially target the most potent antigen presenting cells, i.e. the dendritic cells. Two prime objectives are currently studied, i) the enhancement of the phagocytosis of DNA-loaded PLGA microspheres by human-derived dendritic cells, and ii) the release of intact plasmid DNA and successful transfection of dendritic cells once the PLGA microspheres have been successfully phagocytosed.

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2. Polymeric Microspheres for Single Injection Vaccination

Vaccine development has traditionally been an almost exclusive business of immunologists, who focused their interest on newest immunological knowledge and biotechnological methods. The search for a larger spectrum of vaccines and improved vaccines was centered on the discovery of new and more immunogenic antigens. The field of vaccinology in a broad sense including formulation aspects received increasing attention only a few years ago. It is now fully recognized that a successful vaccine may depend on the particular delivery route, delivery kinetics, formulation and antigen stability. Therefore, polymers and polymeric delivery systems play an increasing role in vaccine development.

Vaccines represent the most cost-effective approach to control and eradicate microbial infections. Concerted immunization programmes have resulted in the eradication of smallpox and poliomyelitis, and effective use of diphtheria (D), tetanus (T), pertussis (P) and measles vaccines have also been associated with a dramatic reduction in the incidence of these diseases worldwide. Interruption of immunization programmes, such as have occurred in Sweden and UK in the sixties, associated with concerns over safety of whole-cell pertussis vaccines, have led to rapid resurgence of diseases. More importantly, failure to complete the multiple booster injections in infant vaccination regimens in a vast majority of the population can have a pronounced effect on disease incidents, as demonstrated by the re-emergence of diphtheria epidemics in Russia and several of the independent republics of the former Soviet Union.

Vaccines are administered as part of the expanded immunization programmes worldwide. The greatest problem faced in the effective delivery of vaccines is that primary immunization requires multiple injections. Further, periodic boosters are often required throughout life to maintain immunity. Current vaccination schedules used in Europe require that all infants must be immunized within the first 15 months from birth with three doses of D, T, P, *Haemophilus influenzae* B poly(saccharide) (Hib) and poliomyelitis antigen (polio) and one dose of measles, mumps and rubella (MMR) antigens. Additional booster doses are required for D, T, polio and MMR vaccines at 3–5 years of age, followed by another booster of D, T and polio at school-leaving age. In addition, each child is also vaccinated

against tuberculosis (BCG) and, more recently in several European countries, against meningitis C. Thus, each child needs to be vaccinated up to eighteen times in order to be considered fully protected against major infectious childhood diseases. In both developed and developing regions of the world a substantial number of infants fail to receive recommended doses of vaccine required for complete protection and full immunity. It is estimated that the cost of vaccination is only 20% due to vaccine itself, the rest being due to transport, storage and health care personnel required for this complex immunization programme. Thus, lowering the total number of vaccine doses needed would reduce both the cost required for vaccination and number of partially immunized individuals.

Combination vaccines based on DTP have provided the foundation of the childhood immunization programme in the last 50 years. The increasing emergence and registration of a number of new vaccines (*e.g.* against hepatitis A, *Haemophilus influenzae* B and meningitis C) makes it logical to attempt to combine them into tetravalent, pentavalent or hexavalent formulations, partly to reduce the number of injections required. Whilst it may seem relatively straightforward to combine existing registered vaccines, it has taken most experienced vaccine manufacturers 5–10 years to develop effective combination vaccines at an enormous cost. Despite the efforts made to date, some combinations have continually proved ineffective in clinical trials. Development of effective combination

vaccines requires significant technical, clinical, regulatory, manufacturing and marketing challenges.

New antigen delivery technologies are considered essential to remedy the limitations of the existing immunization regimens and to allow for the development of new and improved vaccines. A major milestone in the development of antigen delivery systems for new and more efficacious vaccines was the use of biodegradable poly(lactide) and poly(lactide-co-glycolide) (PLA/PLGA) microspheres (MS), within a special WHO Programme for Vaccine Development, initiated in the late 1980s [2]. PLA/PLGA-MS were expected to release encapsulated antigens in a continuous or pulsatile manner, thereby mimicking the repeated injections of conventional vaccination schedules.

Early developments of PLGA-based vaccines were basically technology-driven stemming from the established biocompatibility of these polymers in concert with their innate properties to tailor rates of bioerosion and release [3]. Pulsatile antigen release patterns have indeed been confirmed for various antigens and PLGA-MS types (Fig. 1). Immunization of small animals such as mice and guinea pigs with a single injection of microencapsulated tetanus or diphtheria toxoid afforded protective and long-lasting antibody responses (Fig. 2). PLGA-MS have also proved their inherent immunostimulating properties for very weakly immunogenic peptide antigens [4]. In future studies, further antigens will be encapsulated individually and, most importantly,

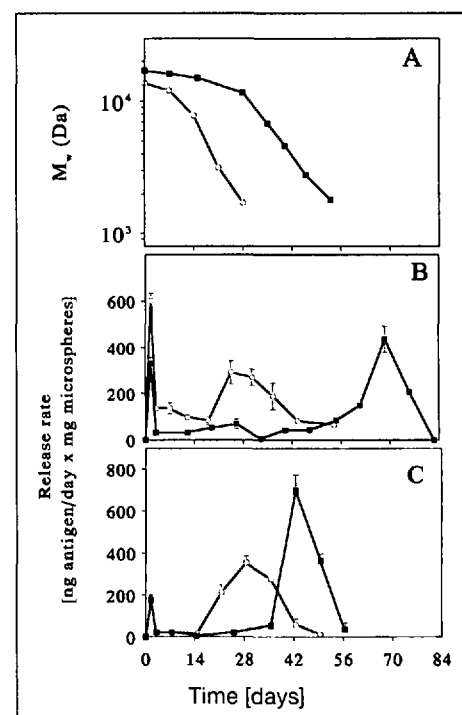


Fig. 1. *In vitro* degradation rate of (A) PLGA 50:50 (○) and PLGA 75:25 (■) microspheres; (B) *in vitro* release profiles of 150 kDa tetanus toxoid, and (C) a 16 kDa antigen, derived from *Plasmodium berghei* circumsporozoite protein, from PLGA 50:50 (○) and PLGA 75:25 (■) microspheres. PLGA ratios indicate molar percentages of lactide to glycolide in polymer.

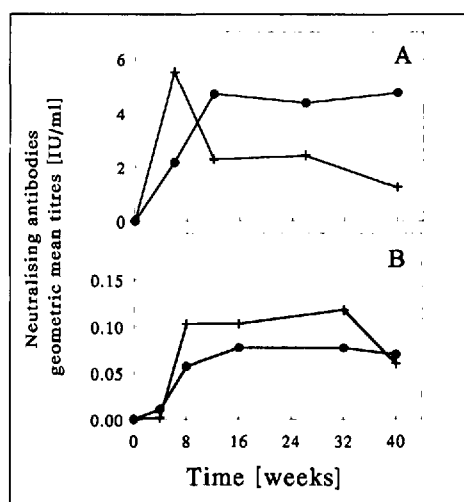


Fig. 2. Neutralizing antibody titers in guinea pigs immunized with tetanus (A) or diphtheria (B) toxoid microencapsulated in PLGA 50:50 microspheres (●) or adsorbed on aluminum hydroxide (alum) (✦). Tetanus toxoid was administered as a single dose of 1 Lf when contained in microspheres, but at 2×0.5 Lf at a four weeks interval when adsorbed on alum (which is the basis of a conventional immunization regimen). Diphtheria was administered as a single dose of 2.5 Lf in both the microsphere and alum formulations. Anti-tetanus and anti-diphtheria antibody titers above 0.01 IU/ml are regarded as protective.

in mixtures to develop a PLGA-based, multivalent single-injection vaccine formulation.

A further most attractive quality of PLGA-MS is their ability to elicit cellular effector responses, *e.g.* so-called cytotoxic T cell (CTL) responses, in addition to the antibody responses observed in the early studies [5][6]. This makes PLGA-MS particularly attractive to fight intracellular infections through bacteria, viruses, and parasites, as well as for cancer vaccination. Moreover, additional features of MS technology (*e.g.* particle size, surface properties, and vehicles for MS administration) can be varied so that they may affect the type and extent of immune response [7]. It is established that the particulate nature of antigens can activate certain antigen presenting cells (APC) such as macrophages and dendritic cells to mediate the presentation of exogenous antigens through MHC-class I molecules, hence to aid in the generation of CTL responses. We have been able to show that macrophages can internalize MS of small size ($< 10 \mu\text{m}$) and then stimulate CTL response *in vitro*. In contrast, the internalization of larger particles is restricted.

Co-entrapment of antigen cocktails, stabilizers, co-adjuvants and other additives in polymeric MS represents additional aspects of MS-based vaccine formulation. Further aspects might be opened by addressing such vaccines to specific cellular targets, *e.g.* by coating with suitable ligands to address the cellular targets. Thus, in addition to the present state of the art, polymeric MS represent a versatile technology to allow the bioengineering of vaccine delivery systems of complex nature and beyond our current anticipation.

3. PLGA Microspheres for Controlled Localized Morphogen Delivery to Enhance Bone Repair

Preservation of bone mass is regulated by a strict control of bone formation and resorption. Systemic and local growth factors guide the underlying processes of bone remodeling. Much effort in tissue engineering is devoted to use this capacity and to identify the therapeutic possibilities to enhance repair and regeneration of durable bone, *e.g.* after fracture, pathology or surgery. As previously reviewed [8] the most prominent approach is the use of various bone morphogenetic proteins (BMPs). This dates back to the mid 1970s when it was discovered that proteins in the natural bone matrix attract stem cells from the bone marrow, then trigger them to proliferate and become bone-producing osteoblasts. The isolation of these proteins took until 1989 when the gene of BMP-7 was cloned and, subsequently, allowed to produce the recombinant version of the protein. To date, BMP-2, BMP-7 and mixtures of BMPs are firmly established to kick start the bone-regeneration process, using different BMP seeded matrices as implants. Another approach to induce bone regeneration includes gene therapy with BMP-2 encoding adenoviral vectors, which were used to transfect bone marrow cells. The transfected cells were grown on a demineralized bone matrix and then surgically implanted to enhance the bridging of critical gaps in the leg bones of test animals. For another gene therapy approach, instead of externally transfecting bone marrow cells, viral vectors encoding human parathyroid hormone (PHT) were trapped in a polymer matrix and directly implanted in the leg bones of dogs.

Full repair of critical gaps was reported. In order to expand the natural recruitment of stem cells at the repair site, further approaches are directed to amplify the supply of natural stem cells with cells grown in culture, namely previously harvested and then cell cultured mesenchymal stem cells (MSC).

In light of the considerable practical problems related to the recombinant production and formulation of BMPs, or those intrinsically associated with the use of gene therapy and/or stem cell technology in a clinical set-up, the need for competitive approaches to induce bone repair is far from saturated. Here we review our current progress on the potential of insulin-like growth factor I (IGF-I) for bone repair. IGF-I is a single chain polypeptide and consists of 70 amino-acid residues. It is secreted by i) production in the liver for endocrine action, and ii) local production, *e.g.* by osteoblasts or chondrocytes, for paracrine action. In fractures, osteoblasts and chondrocytes are responsible for the formation of fracture callus. Thus, IGF-I seems to play an important role in local bone formation and bone repair after injury. Moreover, IGF-I is expected to accelerate the normal healing of bone defects, particularly in cases of heavily impaired healing. In sharp contrast to such expectations, however, were past investigations using local IGF-I administration which were unsuccessful to promote healing of bone defects. A potential explanation for this failure might be that the delivery regimen used may have been inadequate to induce bone repair.

Consequently, control of local IGF-I release kinetics may be a key parameter for therapeutic success. Controlled protein release is typically achieved with biodegradable implants. For instance, biodegradable microspheres may deliver an encapsulated drug at specific body sites over prolonged time periods, thereby providing clinically relevant local doses with appropriate kinetics. This is of particular importance for drugs with a short half-life and/or important systemic toxicity. However, the preservation of full biological activity of incorporated drugs during storage and over the life time of biodegradable delivery systems once injected remains a major challenge.

Our current project in this area aims at developing a polymeric IGF-I drug delivery system for localized administration in bone defects. For preparation of IGF-I loaded PLGA microspheres (microencapsulation) we used a double emulsion solvent evaporation technique. The biological activity of released IGF-I was

confirmed using an *in vitro* fat cell assay. To stabilize IGF-I during and after MS entrapment, a number of suitable biocompatible additives were evaluated [9]. The therapeutic benefit was assessed in a preclinical study in sheep with surgically induced bone defects which were filled with PLGA microspheres containing IGF-I. In addition to evaluating the effect of IGF-I on bone morphology, we investigated the effect of localized IGF-I delivery on mRNA expression of growth factors, proinflammatory cytokines and enzymes to induce inflammatory mediators after one, two, and three weeks postoperatively. The delivery system was designed to release IGF-I in a controlled fashion within a period of seven to fourteen days. Stability and biological activity of IGF-I upon microencapsulation was maintained.

The controlled localized delivery of IGF-I from PLGA microspheres stimulated rapid new bone formation in the bone defect model in sheep. Furthermore, it appeared to affect mRNA expression of bone growth factors, proinflammatory cytokines and of enzymes responsible for the production of mediators. Thus, IGF-I seems to stimulate new bone formation with concomitant changes in the expression of molecules which have been proven important in bone homeostasis. Detailed mechanistic aspects are currently under investigation. In conclusion, the developed microspheres proved to be suitable for the localized delivery of intact IGF-I over up to thirteen days, a time period considered to be relevant to enhance bone formation. Moreover, we have preliminary indications that in sheep critical bone gaps can be successfully bridged within short time. Relative to the other approaches in bone repair and regeneration, namely the BMPs, gene therapy and stem cell technology, the controlled localized delivery of IGF-I entrapped in PLGA microspheres appears to be highly competitive.

4. PLGA Microspheres to Target Dendritic Cells for Genetic Engineering

Dendritic cells (DCs) are antigen-presenting cells with a unique ability to induce primary immune responses [10]. They occur in various tissues in an immature state and are able to capture both soluble and particulate antigens which are subsequently targeted to a class I or class II presenting pathway. DCs have been demonstrated to play a central role in controlling immunity and represent the most potent antigen-presenting cells currently known. Protective immunity against viral infections as well as tumor protection was most efficiently restored by administration of antigenic peptides or tumor antigens *via* DCs, rather than in combination with adjuvant [11][12]. This underlines the importance of the direct targeting of DCs to affect immunity.

DNA vaccines have emerged as a realistic alternative for preventive or therapeutic immunization [13]. The application of genetically engineered DCs is especially appealing since it offers the opportunity to express an ever-increasing number of antigens as yet unidentified peptide epitopes in addition to chemokines and cytokines that modulate T cell responses.

Non-viral or synthetic gene delivery systems have emerged as a promising technology to circumvent safety concerns associated with the use of replication-competent viral vectors. A suitable synthetic gene delivery system should serve both i) protection of a gene expression system from premature degradation in the biological milieu and ii) adequate

non-specific or cell-specific delivery to the target cell. Other elements of the gene delivery system may facilitate the intracellular trafficking of a gene expression system and its translocation to the nucleus, where gene expression can occur (Fig. 3).

Biodegradable microspheres based on PLGA have shown promising results in the delivery of antigens to antigen-presenting cells and in stimulating potent immune responses [14]. Small size PLGA microspheres are internalized efficiently by phagocytes such as macrophages. Thus, they are capable of delivering the encapsulated antigen to the major histocompatibility class I pathway indicating access to the cytosol of the target cells [14]. In addition, due to their size in the micrometer range, microspheres are not internalized by the majority of the other cell types and are suited to specifically target phagocytic cells. These findings stress the importance of investigating PLGA microspheres as gene delivery systems to DC.

We are currently studying the influence of different PLGA type polymers on the encapsulation and release of DNA and have identified a rather hydrophilic low molecular weight PLGA polymer to provide a suitable carrier material for DNA containing microspheres. Stabilization of DNA during the encapsulation was achieved by adding a buffering substance prior to emulsification of the aqueous phase and the subsequent spray-drying process [15]. We achieved small particles with high loading efficiency and with the majority of the DNA remaining in its supercoiled, most stable and active topological form (Fig. 4, Table).

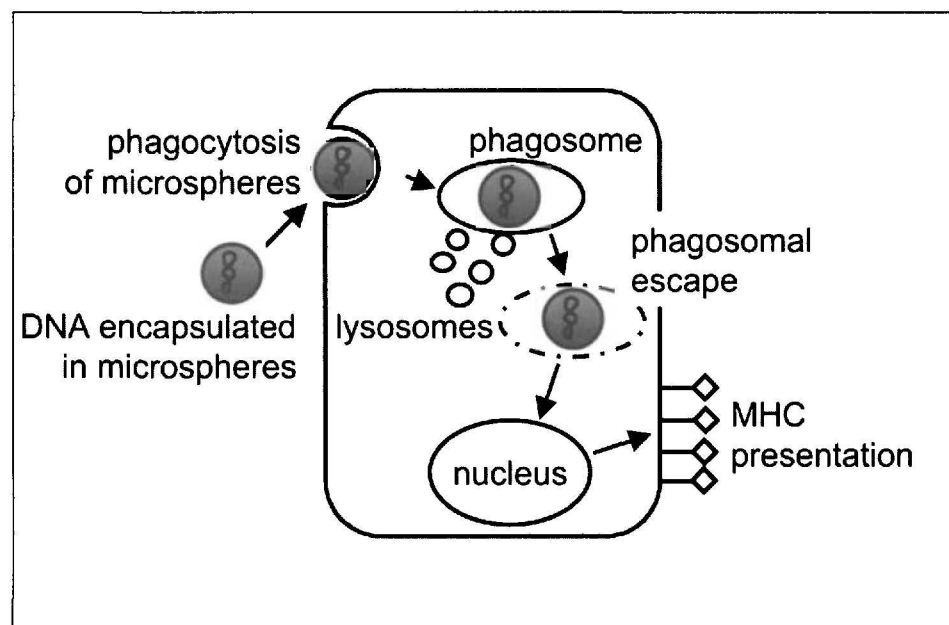


Fig. 3. Critical steps for the genetic engineering of dendritic cells (DC) by plasmid DNA-loaded PLGA microspheres.

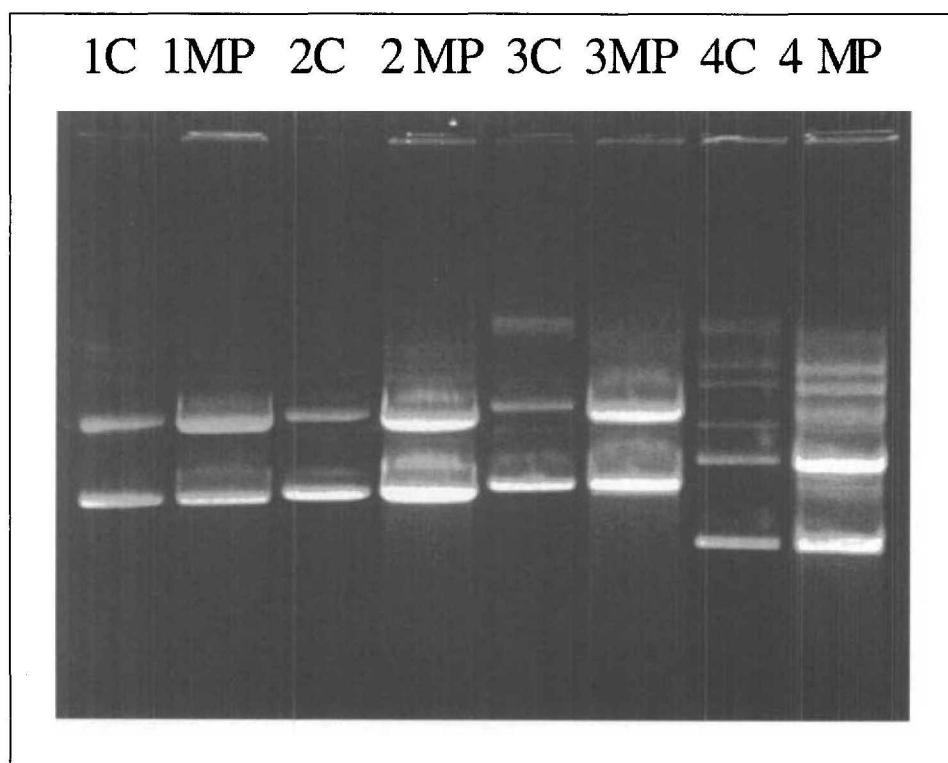


Fig. 4: Stability of DNA entrapped in PLGA microspheres. Agarose gel demonstrating different plasmids (1-4) encapsulated in PLGA microspheres. The lower band represents supercoiled DNA and the upper band open circular DNA. C = plasmid control, MP = plasmid extracted from PLGA microspheres.

Table. Encapsulation of plasmid DNA in PLGA microspheres

Plasmid	Loading [$\mu\text{g}/\text{mg}$]	
	Total DNA	Double-stranded DNA
1	9.64	8.30
2	12.3	9.36
3	11.6	13.7
4	11.0	14.6

It has been demonstrated that DC are able to capture antigens, whole microbes, apoptotic bodies, and to a certain extent also latex microparticles [10]. Previously we showed PLGA microspheres to be efficiently taken up by human DCs, leading to the delivery of high amounts of encapsulated DNA into DC. All investigated PLGA microsphere preparations were fully biocompatible and there were no significant apoptotic or necrotic events after phagocytosis after up to one week incubation. Moreover, we showed that intracellular degradation of microparticles is dependent on the type of PLGA-polymer employed and can be chosen in a controlled manner (Fig. 5).

We have demonstrated that biodegradable microspheres deliver encapsulated DNA to phagocytic DCs in large quantities and DNA is released inside the cells upon biodegradation of the microspheres. However, gene expression was

very low and may be restricted by phagolysosomal entrapment of the DNA (Fig. 3). Future work is therefore focused on the identification of possible mechanisms to escape phagolysosomal degradation and gain access to the cytosol of DCs. Phagocytic cells generally contain phagosomes and lysosomes which are numerous and large in size underlining the potent scavenger function of these cells. Furthermore, endosomal disrupting agents act in a concentration dependent manner and will probably require a high local concentration of the agent to reach the membrane disrupting threshold. This is likely to act as a bottleneck for efficient genetic engineering of DCs. Numerous membrane disrupting agents, such as peptides, amphiphilic molecules and polymers have been identified and may help to increase the transfection efficiency. For this purpose, biodegradable microspheres provide an excellent carrier sys-

tem for the co-delivery of the respective membrane disruptive compound in sufficient amounts together with the plasmid DNA. The release pattern of both components may be controlled by the type of polymer and the encapsulation technique and can be scheduled simultaneously or individually.

In summary, gene delivery systems are generally designed to overcome various barriers such as transport through the cell membrane into the cells, endosomal/lysosomal escape and transport into the nucleus. Biodegradable polymeric microspheres can be used to selectively target phagocytic cells such as DCs and deliver encapsulated DNA in large quantities into the cells. We feel that the transfer of DNA out of the phagosomal compartment may be one of the most critical steps in genetic engineering of DCs.

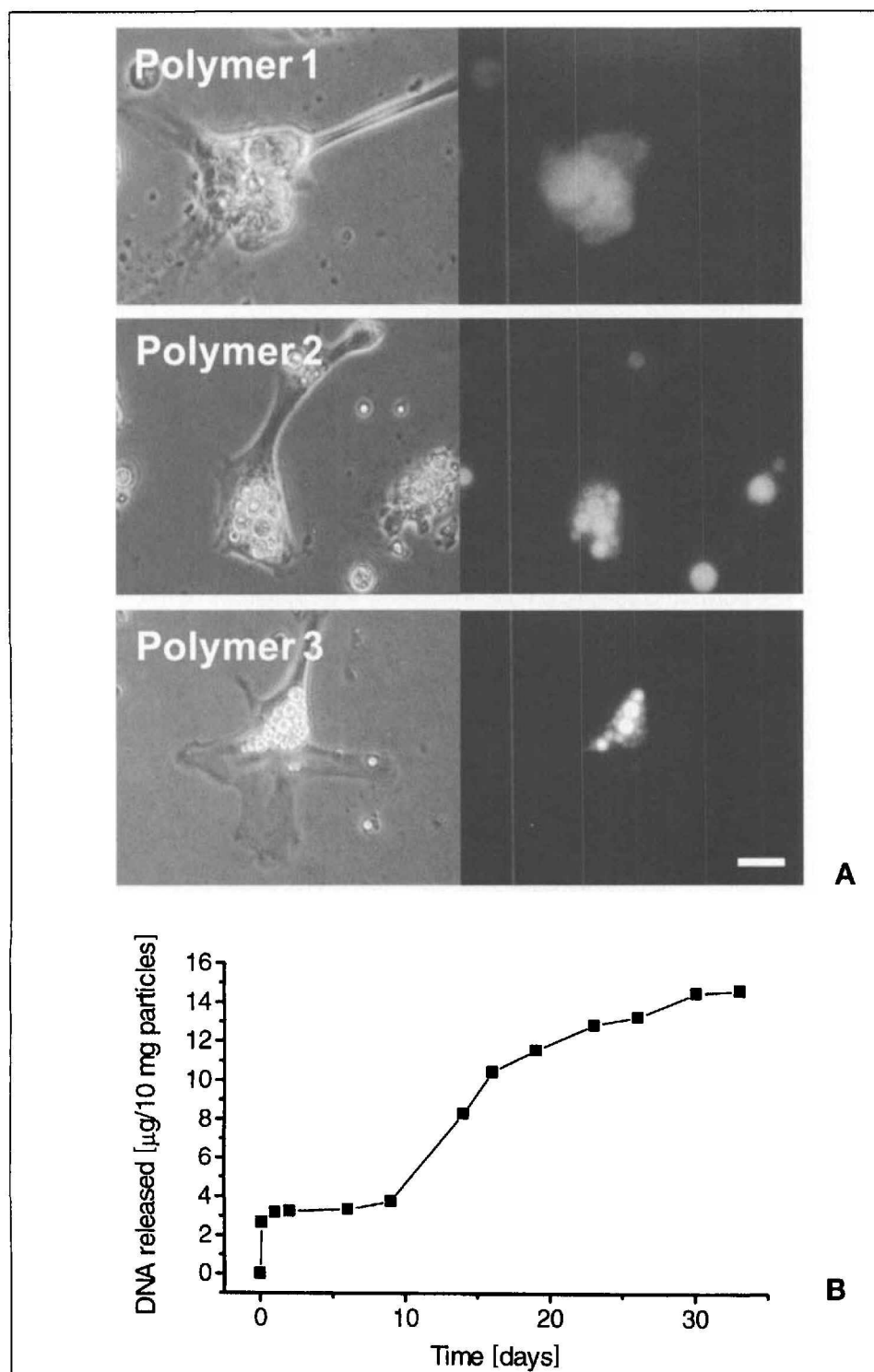


Fig. 5. (A) Fluorescence-labelled microspheres from three different PLGA-type polymers 13 days after uptake by human derived DCs. Cells were examined simultaneously by phase contrast (left panel) and fluorescence microscopy (right panel). Only microspheres from polymer 1, a hydrophilic end-group uncapped PLGA, are biodegraded in the cells, whereas PLGA polymers 2 and 3 remain intact within the studied time frame. Bar = 20 μm . (B) *In vitro* DNA release from PLGA microspheres (polymer 1) in phosphate buffered saline at pH 5.8. Samples were analyzed for double stranded DNA.

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