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Materials in particulate form for tissue engineering. 1. Basic concepts

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Materials in particulate form for tissue engineering. 1. Basic concepts

Abstract
For biomedical applications, materials small in size are growing in importance. In an era where 'nano' is the new trend, micro- and nano-materials are in the forefront of developments. Materials in the particulate form aim to designate systems with a reduced size, such as micro- and nanoparticles. These systems can be produced starting from a diversity of materials, of which polymers are the most used. Similarly, a multitude of methods are to produce particulate systems, and both materials and methods are critically reviewed here. Among the varied applications that materials in the particulate form can have, drug delivery systems are probably the most prominent, as these have been in the forefront of interest for biomedical applications. The basic concepts pertaining to drug delivery are summarized, and the role of polymers as drug delivery systems conclude this review.

Keywords
microparticles, nanoparticles, drug delivery, tissue engineering, polymers, ceramics, natural origin

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Materials in particulate form for tissue engineering.  
1. Basic concepts

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Abstract

For biomedical applications, materials small in size are growing in importance. In an era where ‘nano’ is the new trend, micro- and nano-materials are in the forefront of developments. Materials in the particulate form aim to designate systems with a reduced size, such as micro- and nanoparticles. These systems can be produced starting from a diversity of materials, of which polymers are the most used. Similarly, a multitude of methods are to produce particulate systems, and both materials and methods are critically reviewed here. Among the varied applications that materials in the particulate form can have, drug delivery systems are probably the most prominent, as these have been in the forefront of interest for biomedical applications. The basic concepts pertaining to drug delivery are summarized, and the role of polymers as drug delivery systems conclude this review.

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particles was recognized years ago by the pharmaceutical industry as a viable means of improving drug delivery (Bissery et al., 1984; Bezemer et al., 2000a, 2000b; Pillai et al., 2001). This use arose because conventional dosage forms, such as oral delivery and injection, were not able to control the rate of delivery or the target area of the bioactive agent and were often associated with an immediate or rapid release (Tao and Desai, 2003).

The main advantages of microparticles is that they may be administered by injection or intranasally as a dry powder, so that a surgical procedure is not required (Baldwin and Saltzman, 1998; Eliaz and Kost, 2000; Tinsley-Brown et al., 2000), and that they may contain a greater amount of biologically active molecules per unit volume (Langer, 1991; Grassi et al., 2001; Janes et al., 2001a). Various parameters, including particle size and distribution, porosity, pore structure and surface area, are considered to describe the overall performance of polymer microparticles in biomedical applications (Tuncel et al., 1996; Allemann et al., 1998; Yang and Alexandridis, 2000). Additionally, the use of microparticles composed of biodegradable polymers eliminates the need for device removal after release of the agent (Baldwin and Saltzman, 1998). Based on these features, microparticles have been the subject of numerous studies with the intent to overcome a number of issues related to the therapeutics of biologically active molecules.

In summary, microparticles have the following properties that render them attractive:

- **Size:** small size allows them to be inserted in the target area in a non-invasive manner, thus increasing effectiveness.
- **Size distribution:** microparticles ranging from a few to a few hundred µm can be selected according to a specific application.
- **Porosity and pore structure:** the presence of pores allows the tailoring of the release profile.
- **Surface area:** large surface area and a capacity for loading the bioactive agent at a high fraction of the total weight of the particle.

However, for some applications, particles with an even smaller size – nanoparticles – can be preferable to microparticles.

### 2.2. Nanoparticles

Nanoparticles, being submicron systems, have the advantage of an even larger surface area compared with microparticles, because the total surface area is inversely proportional to the third power of the diameter (Berton et al., 1999; Kawaguchi, 2000). In these systems the bioactive agent can be dissolved, entrapped, encapsulated, adsorbed, immobilized or attached to the matrix (Orive et al., 2004) and, depending upon the method of preparation, nanoparticles, nanospheres or nanocapsules can be obtained (Couvreur and Puisieux, 1993; Soppimath et al., 2001). Nanocapsules are vesicular systems in which the bioactive agent is confined to a cavity surrounded by a unique polymer membrane, while nanospheres are matrix systems in which the bioactive agent is physically and uniformly dispersed (Soppimath et al., 2001). Nanospheres and nanocapsules are the morphological equivalents of microspheres and microcapsules, respectively (Allemann et al., 1998).

Nanoparticles can be injected and, as a result, can circulate in the blood stream (Madan et al., 1997). However, in some cases, nanoparticles are phagocytosed by macrophages (Lee et al., 2001), and this can lead to an adverse immunological response. However, such reaction may be desirable in applications such as vaccination therapies, and when enhanced uptake of exogenous compounds, such as anti-human immunodeficiency virus (HIV) drugs (Lee et al., 2001), is sought. Nanoparticle polymeric carriers, when their size is less than 100 nm, have a high potential for being accumulated in tumour sites, according to the enhanced permeation and retention (EPR) effect (Nishikawa et al., 1996; Yasugi et al., 1999). Hydrophilic modification, particularly by introducing poly(ethylene)glycol (PEG) by physical coating or covalent linking – a process known as pegylation – to the surface, prolongs the half-life of the carriers (Kumar, 2000; Seal et al., 2001; Diwan and Park, 2003) during circulation in blood by reducing opsonization and thus minimizing carrier clearance in organs such as liver, spleen, lung and bone marrow (Gref et al., 1994; Peracchia et al., 1997). This long-circulating stealth characteristic of the carrier produces the EPR effect, which is valuable in passive cancer targeting (Berthold et al., 1998; Maeda et al., 2000).

Nanoparticles hold great potential for the treatment of tumours. An example is related to the ability of those materials to include within their matrix magnetic particles and by directing nanoparticles to the target (e.g. tumour cells) through magnetic fields created around the tumour. This brings great advantages, such as a reduction of the dosage and side-effects, as well as a rise in the therapeutic effect, together with controlled and, most importantly, direct targeting of the tumour site (Brigger et al., 2002).

Nanoparticles offer other specific advantages over liposomes, because they increase the stability of bioactive agents/proteins and possess a better set of controlled release properties (Jain, 1994; Hrkach et al., 1997; Gaspar et al., 1998; Berton et al., 1999; Kumar, 2000; Soppimath et al., 2001).

To summarize, nanoparticles possess the following advantages:

- **Stability:** increased stability over liposomes and promotion of increased stability of entrapped bioactive molecules.
- **Surface area:** higher surface area, even when compared with microparticles.
- **Size:** depending on their size, they can be phagocytosed or can circulate in the blood long enough to promote the therapeutic effect.
3. Overview of synthesis methods

There are several methods for the production of micro- and nanoparticles, but the most widely used techniques are methods based in emulsions, such as suspension polymerization, solvent evaporation and, to a smaller extent, organic phase separation (coacervation) and spray-drying methods, as reviewed/described in detail in the literature (Kreuter, 1991; Gref et al., 1994; Tuncel et al., 1996; Madan et al., 1997; O'Donnel and McGinity, 1997; Lin and Yu, 2001; Soppimath et al., 2001).

In suspension polymerization, the monomer phase is broken into droplets (a few µm in diameter) within a dispersion medium (usually an aqueous phase) and stabilized by a surfactant dissolved in the medium (Piskin et al., 1993). These monomer droplets containing a monomer phase soluble initiator are then individually polymerized by applying a temperature/ agitation programme (Piskin et al., 1993). In the emulsion/solvent evaporation method, the polymer is solubilized/dispersed in an organic solvent (e.g. methylene chloride, chloroform) and the resultant solution is then emulsified with an aqueous phase (Soppimath et al., 2001; Perez et al., 2002). The formation of the particles is achieved by hardening resulting from the evaporation of the organic solvent. Stirring speed is usually the parameter controlling the size of the particles. This method is easy to implement and yields very good results with a variety of raw materials.

Most of the methods for the production of particle-based systems are actually based on the creation of emulsions between organic and aqueous phases, and suffer one common drawback—the need for organic solvents (e.g. methylene chloride, chloroform, acetonitrile, tetrahydrofuran) in at least one of the production steps (Ghaderi et al., 1999; Kim and Park, 1999; Sendil et al., 1999; Birnbaum et al., 2000).

The residual content of the organic solvent in the microparticles after preparation has to be removed in time-consuming drying steps (Nykamp et al., 2002), and in many cases the presence of an organic solvent can lead to loss of the activity of the agent to be loaded into the system. Currently, methods that obviate the use of organic solvents are in demand, and this aspect is particularly critical when there is a risk of hindering the activity of the biological agent. An interesting new approach in efforts to address this particular issue is that described by Nykamp et al. (2002), who used a jet-milling technique to produce polylactic acid (PLA) and polyactic/glycolic acid (PLGA) microparticles with different ratios of the two polymers. Conceivably, this method could also be used for other polymers. However, the first step of this process involves melting the starting material, which obviously has to be taken into account when aiming to use the developed systems for delivery of bioactive agents. Similarly, Lin et al. (1999) have used a solvent-free method to produce polycaprolactone (PCL) microparticles, by dispersing polyethylene glycol (PEG) in the PCL phase. Although the melting temperature of PCL is low (close to 60 °C), this temperature might still be deleterious for the activity of bioactive molecules.

One has to be cautious in choosing the method of production, and weigh carefully between the risks of using an organic solvent or using high-temperature conditions, two major parameters influencing the biological activity of an agent.

Although micro- and nanoparticles can be produced using a vast array of possible techniques, a number of variables that affect the product obtained have to be taken into account when choosing a material and method. These include (Bissery et al., 1984; Ronneberger et al., 1997; Bezemer et al., 2000a):

- Type and amount of material used.
- Degradation rate of the polymer.
- Type and payload of bioactive agent being incorporated (in case of drug delivery applications).
- Organic solvent being volatilized.
- Type and amount of surfactant dissolved in the aqueous phase.
- Temperature.
- Pressure during solvent evaporation.
- Ratio of the volume of organic solvent: volume of aqueous phase.

By ‘playing’ with these parameters, researchers have been able to use a wide array of materials and methods for a number of applications.

4. Materials used in the synthesis of materials in particulate form

The polymeric class of materials has been regarded as the primary choice for applications in which small-sized particles are needed, since many polymers can be formed into microparticles and nanoparticles for delivery and other applications. These may be non-degradable or degradable polymers, from synthetic or natural origin, or even blends (synthetic–synthetic, synthetic–natural, or natural–natural). Nevertheless, polymers are not the only materials used for producing materials in particulate form, and across the literature there is a wide array of materials used for the synthesis of particle-based materials, including ceramics and metals. This review deals primarily with polymers and to some extent ceramics. Some examples of polymer–ceramic composites will also be described.

Table 1 summarizes the most frequently used materials for the synthesis of materials in particulate form, and also includes the methods for production of these systems and intended applications, with a brief description of the most widely used groups following the table.
Table 1. Overview of the materials and methods used for the production of materials in particulate form and envisioned applications (information compiled in the scope of this review)

<table>
<thead>
<tr>
<th>Material</th>
<th>Type</th>
<th>Method</th>
<th>Application</th>
<th>Description</th>
<th>Ref.</th>
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<tbody>
<tr>
<td>Synthetic polymers and blends</td>
<td>Microspheres</td>
<td>o/w solvent evaporation</td>
<td>Incorporation and release</td>
<td>Release of epidermal growth factor (EGF)</td>
<td>(Herrmann and Bodmeier, 1995; Delie et al., 2001; Han et al., 2001; Dalpiaz et al., 2002; Tamura et al., 2002; Katare et al., 2005)</td>
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<td>Polylactic acid (PLA)</td>
<td>Polyspheres</td>
<td>Solvent evaporation</td>
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<td>Release of somatostatin</td>
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<td>Double emulsion technique</td>
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<td>Release of cisplatin</td>
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<td>Delivery of antisense oligonucleotides</td>
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<td>Release of the antiischaemic drug</td>
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<td>Release of N6-cyclopentyladenosine</td>
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<td>Entrapment of tetanus toxoid for immunization</td>
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<tr>
<td>Polylactic acid/polyethylene glycol (PLA/PEG)</td>
<td>Micro and nanoparticles</td>
<td>Emulsion–solvent evaporation</td>
<td>Incorporation and release</td>
<td>Release of cyclosporine A</td>
<td>(Gref et al., 2001)</td>
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<td>Polylactic (PLGA)</td>
<td>Microspheres</td>
<td>Water-in-oil-in-water o/w emulsion solvent evaporation</td>
<td>Incorporation and release</td>
<td>Release of active lysozyme</td>
<td>(Herrmann and Bodmeier, 1998; Cruaud et al., 1999; Abazinge et al., 2000; Lam et al., 2000; Perez et al., 2002; De Rosa et al., 2003; Perugini et al., 2003; Jollivet et al., 2004; Wang et al., 2004; Norton et al., 2005)</td>
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<td>Double emulsion (w/o/w) solvent evaporation</td>
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<td>Release of dexamethasone (DEX) and vascular endothelial growth factor (VEGF)</td>
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<td>Microspheres</td>
<td>ProLease® and spray freeze-drying</td>
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<td>Release of prirflavone (for osteopenia treatment)</td>
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<td>Release of enoxacin</td>
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<td>Release of somatostatin</td>
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<td>Release of human IgG</td>
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<td>Release of recombinant human GDNF</td>
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<td>Release of βFGF</td>
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<td>Release of peptide for spinal stenotic disorder</td>
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<td>Release of parathyroid hormone (PTH)</td>
<td>(Meinel et al., 2001; Singh et al., 2001b; Carrascosa et al., 2004)</td>
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<td>Release of gentamicin</td>
<td>(Isobe et al., 1996; Yamazaki et al., 1996; Isobe et al., 1999; Walter et al., 1999; King and Patrick, 2000; Kim and Park, 2001; Ain et al., 2002; Hedberg et al., 2002; Murillo et al., 2002; Zhu et al., 2002; Diwan and Park, 2003; Jalón et al., 2003; Perets et al., 2003; Sanchez et al., 2003; Schlapp and Fries, 2003; García Del Barrio et al., 2004; Matzelle and Babensee, 2004; Siepmann et al., 2004; Wei et al., 2004; Tatard et al., 2005)</td>
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<td>Release of nerve growth factor (NGF)</td>
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<td>Gene transfer via adenovirus</td>
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<td>Release of 5-fluorouracil</td>
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<td>Adjuvant in for immune response</td>
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<td>Release of acyclovir (for Herpes simplex I)</td>
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<td>Encapsulation of Brucella ovis antigens for immunization</td>
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<td>Encapsulation of Helicobacter pylori lysates for immunization</td>
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<td>Release of bone morphogenetic protein (BMP)</td>
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<td>Release of VEGF</td>
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<td>Materials in particulate form for tissue engineering</td>
<td>Microspheres</td>
<td>Microparticles</td>
<td>Nanoparticles</td>
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<td>PLGA/poly(acryloyl hydroxyethyl starch)</td>
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<td>PLGA/εPCL Microparticles</td>
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<td>Poly(L-lactic-co-glycolic acid) and polyethylenoxide (PLGA-PEO-PLGA)</td>
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<td>Poly(γ-benzyl L-glutamate)-poly(ethylene oxide) (PBLG-PEO)</td>
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<td>Poly(acrylic acid) (PAA) Microparticles</td>
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<td>Polyisohexylcyanoacrylate (PIHCA)</td>
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<td>Poly(MePEGcyanoacrylate-co-hexadecylcyanoacrylate) Nanoparticles</td>
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<td>Poly-(isobutyl-cyanoacrylate) (PIBCA) Nanoparticles</td>
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Table 1. (Continued)

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<th>Method</th>
<th>Application</th>
<th>Description</th>
<th>Ref.</th>
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<td>Polymethyl methacrylate (PMMA)</td>
<td>Microparticles</td>
<td>o/w solvent evaporation</td>
<td>Entrapment and release</td>
<td>Release of verapamil</td>
<td>(Streubel et al., 2002)</td>
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<td></td>
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<td>Dispersion polymerization</td>
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<td>Delivery of HIV-1 Tat protein for vaccination applications</td>
<td>(Fundueanu et al., 2001; Caputo et al., 2004)</td>
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<td>Suspension radical co-polymerization</td>
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<td>Buformin tosylate – a classical hypoglycaemic drug</td>
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<td>Poly(methacrylic acid-diethylene glycol) P(MAA-g-EG)</td>
<td>Microparticles</td>
<td>Free-radical solution polymerization</td>
<td>Entrapment and release</td>
<td>Release of insulin</td>
<td>(Morishita et al., 2002)</td>
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<td>Poly(trimethylene carbonate)-poly(ethylene glycol)-poly (trimethylene carbonate) (PTC-PEG-PTC)</td>
<td>Microparticles</td>
<td>Dialysis</td>
<td>Incorporation and release</td>
<td>Release of methotrexate (anticancer drug)</td>
<td>(Zhang and Zhuo, 2005)</td>
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<tr>
<td>Polynylpyrrolidone (PVP)</td>
<td>Nanoparticles</td>
<td>Polymerization</td>
<td>Carrier for antigen</td>
<td>Delivery of the antigen of <em>Aspergillus fumigatus</em> for immune system response</td>
<td>(Madan et al., 1997)</td>
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<td>Polyvinyl alcohol (PVA/P/VAc)</td>
<td>Microparticles</td>
<td>Suspension polymerization</td>
<td>Embolic materials</td>
<td>Introduced through catheters in the management of gastrointestinal bleeders, traumatic rupture of blood vessels</td>
<td>(Lyoo et al., 2002)</td>
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<td>Nanoparticles</td>
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<td>Poly(diethylaminoethyl-ethylene glycol) ε-Poly(caprolactone)</td>
<td>Microparticles</td>
<td>Reverse micelle solvent evaporation</td>
<td>Incorporation and release</td>
<td>Release of superoxide dismutase</td>
<td>(Dubertnet et al., 1987; Pérez et al., 2000; Gibaud et al., 2002a, 2002b; Le Ray et al., 2003; Schaffazick et al., 2003; Youan, 2003; Gibaud et al., 2004)</td>
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<td>Simple and double emulsion–solvent evaporation</td>
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<td>Release of nitrofurantoin (antibacterial agent)</td>
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<td>Release of vancomycin</td>
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<td>Release of fludrocortisone acetate for hormonal therapy</td>
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<td>Release of diclofenac</td>
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<td>Nifedipine (calcium antagonist) and propranolol HCl (β-blocker), for treatment of hypertension</td>
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<td>Melanoprol for the treatment of human trypanosomiasis</td>
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<td>Release of 3,4-diaminopyridine (3,4-DAP) for multiple sclerosis and Lambert–Eaton myasthenia syndrome</td>
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<td>Poly-ε-caprolactone/poly(methyl methacrylate)</td>
<td>Microparticles</td>
<td>Suspension polymerization</td>
<td>N.A.</td>
<td>N.A.</td>
<td>(Abraham et al., 2002)</td>
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<td>Poly-ε-caprolactone/poly(ethylene glycol)</td>
<td>Microparticles</td>
<td>Polymerization and precipitation</td>
<td>Encapsulation and release</td>
<td>Release of all-trans-retinoic acid</td>
<td>(Jeong et al., 2004)</td>
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<td>Double emulsion followed by spray drying</td>
<td>Incorporation and release</td>
<td>Nasal immunization with diphtheria toxoid</td>
<td>(Somavarapu et al., 2005)</td>
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<td>Polytetene</td>
<td>Microparticles</td>
<td>Emulsion solvent evaporation</td>
<td>Incorporation and release</td>
<td>Release of ibuprofen</td>
<td>(Tamilvanan and Sa, 2000a, 2000b)</td>
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<td>Release of indomethacin</td>
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<td>Adjuvant for immune response</td>
<td>(Matzelle and Babensee, 2004)</td>
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N.A., information not available; o, oil; w, water.
The use of synthetic polymers as carriers has predominantly focused on polyhydroxyalkanoates (Ueda et al., 2000; Pillai and Panchagnula, 2001), in particular poly(α-hydroxy esters), because the material has long been used in sutures (Hollinger et al., 1996; Hollinger and Leong, 1996). The most widely used poly(α-hydroxy ester) polymers for particle-based strategies are polylactide (PLA), polyglycolide (PGA) and their co-polymers (poly-ε-lactide-co-γ-glycolide) (PLGA) (Brekke, 1996; Hollinger and Leong, 1996; Whang et al., 1998). Their widespread use stems from the ability of these materials to serve a multitude of purposes and applications.

PLA nanoparticles, in general, have the advantage to be able to pass through the capillary bed and to be mainly concentrated in the liver (60–90%), spleen and lungs (2–10%) and, to a lesser degree, blood marrow (Kreuter, 1983; Brannon-Peppas, 1995). For PLA nanoparticles injected subcutaneously or intramuscularly, they are able to reside at the injection site until biodegradation yields a certain critical molecular weight that enables removal of the degradation products (Kreuter et al., 1983). These particular traits render these systems very interesting for drug delivery applications. Furthermore, tuning of the biodegradability can be performed by blending PLA and PGA in a co-polymer (PLGA), and by changing the proportion of each of these materials in the co-polymer (Miller et al., 1977; Pillai and Panchagnula, 2001; Grayson et al., 2004), as PLA degrades much slower than PGA. Degradation of PLA and PLGA is known to proceed by hydrolytic scission of the polymer chain and depolymerization is influenced by molecular weight (MW), polydispersity and crystallinity (Weinhold et al., 1998; Li and Wozney, 2001).

Although PLGA represents the ‘gold standard’ exemplified by more than 500 patents of biodegradable polymers, increased local acidity because of breakdown products of these polymers can lead to irritation at the target site and may also be detrimental to the stability of protein bioactive agents (Pillai and Panchagnula, 2001). Additional potential problems with these synthetic materials include poor clearance – particularly for high MW polymers – and chronic inflammatory response (Kirkert-Head, 2000; Li and Wozney, 2001). For this reason, research has been focusing on other synthetic materials, such as poly(ε-caprolactone) (ε-PCL), which was, for instance, found to meet the requirements of a biodegradable reservoir or monolithic device for controlled drug delivery, especially in the contraceptive field (Pitt et al., 1979; Dubertret et al., 1987).

Polyorthoesters (POE) have been under development since the 1970s, and they are unique among all biodegradable polymers, as choosing appropriate diols or mixture of diols in their synthesis can readily vary many of their properties. A number of applications have been found for this class of polymers, such as delivery of 5-fluorouracil, periodontal delivery systems of tetracycline and pH-sensitive polymer systems for insulin delivery (Zignani et al., 2000; Pillai and Panchagnula, 2001).

Polyanhydrides have been considered to be useful biomaterials as carriers of bioactive agents to various organs of the human body, such as bone tissue, blood vessels, brain and eyes (Kumar et al., 2002). They can be prepared easily from readily available, low-cost resources, can be manipulated to meet desirable characteristics, are biocompatible and degrade in vivo into non-toxic diacid counterparts that are eliminated from the body as metabolites (Kumar et al., 2002).

However, synthetic materials do not completely fulfill current needs in terms of biomedical applications, and in recent years many researchers have been turning their research focus to materials of natural origin, as these might obviate several of the drawbacks of synthetic materials.

Polyaminoacids, such as poly(γ-methyl-l-glutamate), that have already shown good biocompatibility, have been investigated for the delivery of low MW compounds (Nathan and Kohn, 1994; Pillai and Panchagnula, 2001). However, their widespread use is limited by their antigenic potentials and some difficulties in the control of release that might arise from the dependence on enzymes for biodegradation.

Collagen, viz. type I collagen, is the most widely used natural polymer and is typically derived from bovine or porcine bone, skin or tendon (Winn et al., 1998). The fact that collagen is of animal origin raises concerns, such as the possibility of transmitting diseases. This is particularly critical for materials from bovine sources, due to malignancies such as bovine spongiform encephalopathy (BSE) and the human variant, Creutzfeldt–Jakob disease (CJD). For this reason, other sources of collagen, such as recombinant forms, are seen as an alternative. Collagen exhibits biodegradability, weak antigenicity and superior biocompatibility (Maeda et al., 1999; Lee et al., 2001). This material is regarded as very promising for the delivery of growth factors, as it was found that an electrostatic interaction was the main driving force for the complexation between acidic gelatin and basic fibroblast growth factor (bFGF) (Lee et al., 2001). Biodegradable collagen-based nanoparticles or nanospheres are thermally stable and readily sterilizable (Rossler et al., 1994; Lee et al., 2001). Moreover, nanoparticles can be taken up by the reticuloendothelial system (Marty et al., 1978) and enable an enhanced uptake of exogenous compounds, such as anti-HIV biologically active agents, by a number of cells, especially macrophages (Bender et al., 1996), which may be an additional advantage of collagen-based nanoparticles as a systemic delivery carrier (Lee et al., 2001). Coupled to a small size and a large surface area, high adsorptive capacity and ability to disperse in water to form a clear colloidal solution, the potential of collagen-based nanoparticles has been demonstrated in their use as a sustained release formulation for anti-microbial agents or steroids (Lee et al., 2001). However, some disadvantages of collagen-based systems include the difficulty of assuring adequate supplies, poor mechanical strength (Fries, 1998) and problems related to the use...
of animal origin (especially bovine) collagen due to
the possibility of disease transmission. Alternatives to
animal origin collagens – those produced by recombinant
technologies – still present a high cost.

*Hyaluronan* (hyaluronic acid), typically derived from
rooster combs, is a minor component of bone extracellular
matrix (ECM) (Li and Wozney, 2001). It has been used
as a carrier for bone morphogenetic proteins (BMPs)
and sodium hyaluronate gel was used as the delivery
system for bFGF (Li and Wozney, 2001). One advantage
of hyaluronic acid is that it is negatively charged and can
form ionic bonds with positively charged BMPs to increase
affinity. Disadvantages of hyaluronic acid include its rapid
resorption unless it is crosslinked or chemically modified
to decrease its intrinsic hydrophilicity (Li and Wozney,
2001).

However, the fear that some of these materials might
additionally be carriers for diseases has led researchers to
find other sources of natural products, mostly originating
from plants and produced by microorganisms. These
might present additional advantages, such as ready
supply, low cost, ability to be processed by several
methodologies and ability to tailor their properties.

In this field of polymers from nature, poly(glucoses),
such as starch and dextrans, have long been used for
encapsulating materials for pharmaceutical, cosmetic or
food applications (Shahidi and Han, 1993; Pereswetoff-
Morath, 1998; Zeller et al., 1999; Engelmann et al.,
2004). *Dextran* s are being actively investigated for
sustained delivery of therapeutic and imaging agents,
particularly for injectables and colon-specific DDSs.

*Starch*-based polymers have been proposed by Reis and
Cunha (1995) as materials with potential for biomedical
applications, particularly as scaffolds for bone tissue
engineering applications (Gomes et al., 2001, 2002),
bone cements (Espigares et al., 2002; Boesel et al., 2003)
and recently as drug delivery systems (Elvira et al.,
2002; Silva et al., 2005). These materials have been
shown to be biocompatible in vitro (Mendes et al., 2001;
Marques et al., 2002), and to possess a good in vivo
performance (Mendes et al., 2003; Salgado et al., 2005).

A very important feature of most natural-origin materials,
besides the ones described above, is the reaction of the
host to degradation products (in the case of starch, the
degradation products are oligosaccharides, which can
be readily metabolized to produce energy). Regarding
their biodegradability, enzymes typically catalyse the
hydrolysis of natural biodegradable polymers, e.g. α-
amylase catalyses the hydrolysis of starch, which may
constitute a strategy to tailor the biodegradability of the
material (Azvedo et al., 2003; Araújo et al., 2004;
Touvinen et al., 2004).

*Chitosans* are promising natural polymers that show
biocompatibility, good absorption-enhancing, controlled
release (Janes et al., 2001a; Mao et al., 2001; Pillai and
Panchagnula, 2001), bioadhesive properties (Pillai and
Panchagnula, 2001), as well as cell culture, enzymatic
immobilization and chromatograph support (Kumar,
2000). Chitosan is a product of the deacetylation of chitin,
produced with varied degrees of deacetylation, and its
use is only limited by the poor solubility or insolubility of
chitosan in water (Wang et al., 2002). However, growing
attention given to this material for several applications,
not only for drug delivery, makes us believe that chitosan
holds promise to become a very successful material for
biomedical applications.

Another widely used polymer of natural origin
is *alginate*, a natural polysaccharide extracted from
brown algae and composed of various proportions of
β-D-mannuronic acid (M) and α-L-guluronic acid
(G) residues. This naturally occurring biopolymer has
many applications in various areas of biosciences and
biotechnology (e.g. as a matrix for the entrapment and/or
delivery of a variety of proteins and cells) and in the
food and beverage industry (as a thickening or gelling
agent and a colloidal stabilizer) (Smidsrød and Skjåk-
Bræk, 1990; Safarikova et al., 2003; Gu et al., 2004).

Besides the best-known method to prepare alginate
beads – which is a gelation method in which a sodium
alginate solution is single-dropped into a calcium solution,
forming particles several μm in diameter – several other
well-known methods (atomization, spraying and water-
in-oil emulsification methods) can also be used to prepare
alginate microparticles that are less than 200 μm in
diameter (Gomboz and Wee, 1998; Safarikova et al.,
2003). Gelation occurs by an ionic interaction between
the calcium ions and the carboxylate anions of G–G
blocks as calcium ions diffuse from the external source
into the droplet (Gu et al., 2004). The main advantage
of using alginate is that the alginate gelation process
occurs under very mild conditions without using high
temperatures or chemical crosslinking agents (Gu et al.,
2004), thus allowing the preservation of the viability and
biological activity of the entrapped cells and other agents,
respectively. However, the application of this system has
been limited by poor mechanical stability. Combining
alginate with other polymers and ceramic materials has
been shown to obviate this feature (Sivakumar and
Punduranga Rao, 2003). Recent studies have described
a dual function of alginate microparticles as carriers
for both cells and drugs, for application in diabetes
(Ricci et al., 2005), an idea that we also propose for
bone tissue engineering applications using starch-based
microparticles (Silva et al., submitted).

Polihydroxybutyrate is a polyester produced as gran-
ules by microorganisms (Fidler and Dennis, 1992; Saito
and Doi, 1994; Jung et al., 2005) and has been widely
studied for tissue engineering applications (Chen and
Wu, 2005), mainly for scaffold materials in combination
with ceramic materials (Doyle et al., 1991; Knowles et al.,
1992, 1993; Li and Chang, 2004; Li et al., 2005) and also
as a vehicle for drug delivery (Koosha and Muller, 1987;
Koosha et al., 1989).

Although polymers are seen as the most versatile class
of materials, other classes have been widely studied for
biomedical applications. Among these are ceramic mate-
rials, which are refractory, polycrystalline compounds,
composed of ionically bonded compounds (de Groot,
5. Applications

Although some applications of materials in particulate form have been mentioned so far, Table 2 lists the major applications of such materials in the biomedical field. By far the greatest field of application for these materials, as found in the literature, is as drug delivery systems (DDS) and a few important principles regarding this field follow.

5.1. Basic concepts in drug delivery

Drug delivery routes are normally four (Langer, 1991; Nitsch and Banakar, 1994): (a) oral, for pills and syrups; (b) rectal; (c) intramuscular or intravenous, for solutions; and (d) topical, as for eye drops. These conventional systems of drug delivery have a major disadvantage, which is that with time the concentration of the bioactive agent decreases to a minimum, leading to the need for a new dose of bioactive agent within a short time interval. Another problem is that the bioactive agent will be distributed systemically throughout the body of the patient (Langer, 1991; Williams, 1998). In general, for oral drug delivery systems, the major problem is the rapid loss of activity of the therapeutic agent in the hostile environment of the stomach (Ponchel and Irache, 1998; Chellat et al., 2000; Grassi et al., 2001). It has also been observed that chemically attaching a bioactive agent to a polymer (bioactive agent–macromolecule conjugate) may alter such properties as its distribution in the body, rate of appearance in certain tissues, solubility or antigenicity (Langer, 1991; Kumar, 2000).

Since oral drug administration remains the easiest and the most comfortable method (Ponchel and Irache, 1998; Chellat et al., 2000; Pillai et al., 2001; Keegan et al., 2003), the microencapsulation of bioactive agents seemed to be an alternative to overcome the problem, allowing their slow release and protection against the acidic and enzymatic gastric environment (Berthold et al., 1998; Chellat et al., 2000). All these were reasons that led to the development of delivery systems, whose aim is to facilitate the dosage and duration of effect of the bioactive agent, causing minimal harm and improving patient compliance (Langer, 1991; Pillai et al., 2001), since they would allow a reduction of the dosage frequency (Kumar, 2000; Pillai and Panchagnula, 2001).

For drug delivery applications, the development of intravenously administered carriers with blood circulation times long enough to continuously deliver bioactive compounds (Gref et al., 1994; Hrkach et al., 1997; Berton et al., 1999; Kumar, 2000), imaging agents or other entities to specific sites of action (Gref et al., 1994) has been a major challenge, since these carriers must possess a set of features compatible with the task they are required to perform. The desired features of such a carrier include (Gref et al., 1994; Soppimath et al., 2001):

Table 2. Major applications of materials in particulate form in the biomedical field (information compiled in the scope of this review)

<table>
<thead>
<tr>
<th>Applications in the biomedical field</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chromatography</td>
<td>(Attebery, 1975; Rocca and Rouchouse, 1976; Fahlvik et al., 1990; Zhang and El Rassi, 1999; Spiegel et al., 2001)</td>
</tr>
<tr>
<td>Imaging</td>
<td>(Cuthbertson et al., 2003; Cavaliere et al., 2005; Huang et al., 2006; Kilbanov, 2006)</td>
</tr>
<tr>
<td>Filling of defects</td>
<td>(Scheppers et al., 1991, Guicheux et al., 1997; Santos et al., 1998; Scheppers et al., 1998; Falaize et al., 1999; Huygh et al., 2002; Day et al., 2004; Domingues et al., 2004; Gossain, 2004)</td>
</tr>
<tr>
<td>Adjuvants in vaccines</td>
<td>(Ohagan et al., 1993; Moore et al., 1995; Nakaoka et al., 1995; Ertl et al., 1996; Heritage et al., 1996; Ohagan et al., 1997; Stertman et al., 2006)</td>
</tr>
<tr>
<td>Cell culture</td>
<td>(Maldia et al., 2003b; Xu et al., 2003; Zhang et al., 2003; Liu and Wu, 2004; Yokomizo et al., 2004; Hong et al., 2005; Melero-Martin et al., 2006)</td>
</tr>
<tr>
<td>Drug delivery</td>
<td>(Herrmann and Bodmeier, 1995; Guicheux et al., 1997; Berthold et al., 1998; Herrmann and Bodmeier, 1998; Jeong et al., 1998; Crusaud et al., 1999; Ganza-Gonzalez et al., 1999; Lam et al., 2000; Lim et al., 2000; Brigger et al., 2001; Delie et al., 2001; Han et al., 2001; Singh et al., 2001a, 2001b; van der Lubben et al., 2001; Delpiaz et al., 2002; Demers et al., 2002; Ko et al., 2002; Morishita et al., 2002; Perez et al., 2002; Tamura et al., 2002; Yenice et al., 2002; Chinen et al., 2003; De Rosa et al., 2003; Perugini et al., 2004; Gu et al., 2004; Jeong et al., 2004; Jollivet et al., 2004; Wang et al., 2004; Horton et al., 2005; Silva et al., 2005)</td>
</tr>
</tbody>
</table>
That the agent to be encapsulated comprises a reasonably high weight fraction (loading) of the total carrier system (e.g., \( >30\% \)).

The amount of agent used in the first step of the encapsulation process is incorporated into the final carrier (entrainment efficiency) at a reasonably high level (e.g., \( >80\% \)).

The ability to be freeze-dried and reconstituted in solution without aggregation.

Biodegradability.

Characteristics to prevent rapid clearance of the particles from the bloodstream.

Also, within drug delivery systems, it is essential to distinguish between sustained and controlled delivery systems, as these two types denote very different applications. Sustained systems imply that the bioactive agent is delivered over a prolonged period of time to overcome the highly periodic nature of tissue levels associated with conventional (enteral or parenteral) administration of single doses by tablets or fluids (Langer, 1991; Silvio et al., 1994; Williams, 1998). The term 'controlled' is used generically to indicate any device in which some control is exerted over the way in which the bioactive agent is delivered to the tissues once it has been administrated to the patient (Langer, 1991; Silvio et al., 1994; Williams, 1998). This is best exemplified in the concept of thermally and pH-responsive materials, where variation in the temperature/pH discontinuously or sharply changes properties such as volume (De Jaeghere et al., 2000; Kawaguchi, 2000; Morishita et al., 2002). This concept is extremely important, as it can be used as a means to trigger the release of the entrapped bioactive agent, and thus allow control to be exerted over the system.

If other ways of controlling the system can be developed, besides temperature and pH, e.g., the presence of a certain agent would trigger the release of the incorporated agent, this could be used for other applications. One such application has been described by Cavanaugh et al. (2001), in which the microparticles released their load of adenovirus only upon cell contact, thus preventing inactivation of the viral load.

5.2. Polymers as the primary choice for DDS

The class of materials that has been most widely studied for drug delivery applications is the polymeric one.

Polymeric delivery systems generally release bioactive agents by the following mechanisms (Langer, 1991; Chellat et al., 2000): diffusion, chemical reaction or solvent activation. The release of a bioactive agent from a matrix is primarily controlled by diffusion of the bioactive agent through the polymer, erosion of the polymer being an additional but important factor (Grassi et al., 2001).

For biodegradable polymers, degradation is a chemical process, whereas erosion is a physical phenomenon dependent on dissolution and diffusion processes. As soon as the bioactive agent-containing polymer (A) comes into contact with the external liquid environment, it enters the polymer matrix (B), resulting in a swelling process (C), which allows the diffusion of the bioactive agent into the external environment (Grassi et al., 2001) (D), as illustrated in Figure 1. Factors influencing the release rate include the molecular size of the bioactive agent and loading percentage into the polymer, as well as polymer composition, molecular weight and the dimensions and shape of the matrix (Langer, 1991).

There are usually three distinct phases of release for biodegradable polymers (as shown in Figure 2):

1. A burst or initial period of rapid diffusion of active agent located close to the surface of the polymer.
2. A period of minimal release, during which the polymer is gradually hydrolysed in bulk but has not yet decreased sufficiently in molecular weight to allow an increased diffusional release of the active agent.
3. The molecular weight of the polymer is sufficiently low as to allow its solubilization in the aqueous environment, and the release of the remaining active agent occurs as the polymer is eroded (Weinhold et al., 1998; Berkland et al., 2002).

This release profile is generally regarded as a problem common to many biodegradable systems, where the release is dependent upon degradation of the system with time (Silvio et al., 1994), thus there is no possibility of achieving any kind of control. This type of device is therefore more suitable for sustained rather than controlled release.

In short, and for drug delivery systems in general, the bulk properties of the polymer that need to be considered include (Langer, 1991; Pillai and Panchagnula, 2001):

- Molecular weight.
- Physical properties (bioadhesiveness, mechanical stability).
- Solubility based on the release mechanism (diffusion or dissolution-controlled).
- Site of action.

Bioadhesiveness needs to be taken into account when drug delivery systems are targeted to mucosal tissues, whereas polymers for ocular devices have to be water- or lipid-soluble in addition to having good film-forming ability and mechanical stability for good retention. The structural properties of the matrix, its micromorphology and pore size, are important with respect to mass transport (of water) into and (of bioactive agent) out of the polymer (Pillai and Panchagnula, 2001).

Of great importance, however, is the assurance that the biological activity of the incorporated agent is preserved throughout manufacturing, storage, delivery and release (King and Patrick, 2000). This, together with the release profile, is of particular importance when designing a delivery system, because much as the release profile may be adequate, there is no point in having it if the biological activity of the agent to be delivered is lost during processing. This idea is mostly coupled with the use of solvents in the production of the delivery system because, as mentioned before, organic solvents might cause inactivation of the agent to be loaded into the system. For growth factors, BSA has been shown to be protective when used as an adjuvant during the loading process (Kim and Valentini, 1997; Morlock et al., 1998), but methods that obviate this step are needed.

Regarding the release profile, strategies to control or render it more adequate for a particular application, by means of modifying parameters such as the surface (by coating, chemical modification) or creating dual-release systems (layers of materials that can incorporate different molecules) (Kim and Valentini, 1997; Vaz et al., 2004), can greatly improve the properties of several materials, and should be actively pursued.

6. Conclusions

Materials in the particulate form have been employed in a diversity of biomedical applications. This derives from their properties, such as size, surface area, and physicochemical properties, which stem from the diverse materials and methods combined for their production. Within the range of applications, drug delivery has had a highlighted role, because of its promise as a means of overcoming limitations inherent to conventional delivery methods. Currently, the use of these systems in innovative strategies, where they can play a multitude of roles – delivery of bioactive agents, structural support and carriers of cells – makes it mandatory for researchers to become even more creative in developing such a system. Within this perspective, an area of tissue engineering that can obviously benefit from the specific properties of materials in particulate form is bone tissue engineering.

Part B of this review (this issue) deals with the roles – played and potential – of particle-based systems in this specific subset of tissue engineering applications, bone tissue engineering.

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