January 2007

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

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Recommended Citation  

Postprint version. Published in Journal of Tissue Engineering and Regenerative Medicine, Volume 1, Issue 1, January 2007, pages 4-24.  
Publisher URL: http://dx.doi.org/10.1002/term.2

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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.

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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³Center for Bioactive Materials and Tissue Engineering, University of Pennsylvania, Philadelphia, PA 19104, USA

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

1. Definition

The key feature of particulate materials systems being their reduced size, the question regarding the threshold size for considering a system to be a particulate one is of value. Across the literature, many authors differ regarding this question. Herein, micron (μm)-sized systems in the range 1–1000 μm will be considered first. Nano-sized particle systems, within this context, are those for which the sizes are below 1 μm (Kreuter, 1991), and they will be described next.

2. Classification of materials in particulate form

2.1. Microparticles

Microparticles consist of particles in a size range 1–1000 μm (Couvreur and Puisieux, 1993). These include microcapsules, vesicular systems in which a cavity is surrounded by a unique polymeric membrane, and microspheres, which are matrix-filled systems (Couvreur and Puisieux, 1993). Polymer microspheres have attracted attention as carrier matrices in a wide variety of medical and biological applications, such as affinity chromatography, immobilization, immunoassay, nuclear imaging and cell culture (Tuncel et al., 1996; Kamyshny and Magdassi, 2000; Shinkai, 2002). Additionally, the incorporation of bioactive agents into small polymeric
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; 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.

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DOI: 10.1002/term
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 poly(lactic/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; Bezemert et al., 2000a):• Type and amount of material used.
• 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; 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.

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DOI: 10.1002/term
## 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 Type</th>
<th>Method</th>
<th>Application</th>
<th>Description</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Synthetic polymers and blends</td>
<td></td>
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</tr>
<tr>
<td>Polyactic acid (PLA)</td>
<td>Microspheres</td>
<td>o/w solvent evaporation</td>
<td>Incorporation and release</td>
<td>Release of epidermal growth factor (EGF)</td>
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<tr>
<td></td>
<td></td>
<td>Solvent evaporation</td>
<td></td>
<td>Release of somatostatin</td>
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<td></td>
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<td>Double emulsion technique</td>
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<td>Release of ciclatin</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></td>
<td>Entrapment of tetanus toxoid for immunization</td>
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<td>Release of cyclosporine A</td>
</tr>
<tr>
<td></td>
<td>Micro and nanoparticles</td>
<td>Emulsion–solvent evaporation</td>
<td>Incorporation and release</td>
<td>Release of active lysoyzme</td>
</tr>
<tr>
<td>Polyactic acid/polyethylene glycol (PLA/PEG)</td>
<td></td>
<td></td>
<td></td>
<td>Release of doxemathasone (DEX)</td>
</tr>
<tr>
<td></td>
<td>Polylactic (PLGA) Microspheres</td>
<td>Water-in-oil-in-water o/w solvent evaporation</td>
<td>Incorporation and release</td>
<td>Release of pririflavone (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 mFGF</td>
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<td></td>
<td>Release of bone morphogenetic protein</td>
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<td></td>
<td>Release of bone morphogenetic protein</td>
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<td></td>
<td></td>
<td>Release of VEGF</td>
</tr>
<tr>
<td></td>
<td>Microparticles</td>
<td>w/o/w–double emulsion–solvent evaporation</td>
<td>Incorporation and release</td>
<td>Release of parathyroid hormone (PTH)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Water-in-oil-in-water emulsion–evaporation</td>
<td>Incorporation and release</td>
<td>Release of gentamycin</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Multiple emulsion solvent evaporation</td>
<td>Incorporation and release</td>
<td>Release of bone morphogenetic protein (BMP)</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td>Release of bone morphogenetic protein (BMP)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>Release of VEGF</td>
</tr>
</tbody>
</table>
Materials in particulate form for tissue engineering. 1

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Encapsulation and release</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microcapsules</td>
<td>Solvent evaporation</td>
</tr>
<tr>
<td>Nanospheres</td>
<td>Water-in-oil-in-water emulsion solvent evaporation</td>
</tr>
<tr>
<td>Nanoparticles</td>
<td>Double emulsion solvent evaporation</td>
</tr>
</tbody>
</table>

- **Release of TP508 from particles as components of composite scaffolds.** TP508 (Chrysalin) 23-amino acid synthetic peptide representing the non-proteolytic receptor-binding domain of thrombin (Hoshino et al., 2000; Takada et al., 2003; Labhasetwar et al., 1999).

- **Release of NF-κB decoy oligonucleotides for inhibition of tumour cell proliferation.** (Humphrey et al., 1997; Song et al., 1997; Gaspar et al., 1998; Mu and Feng, 2002).

- **Release of interferon-α (treatment of hepatitis C).**

- **Delivery of antitubercular drugs.**

- **Release of human growth hormone.**

- **Release of TAK-778 (Hoshino et al., 2000; Takada et al., 2003).**

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Incorporation and release</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLGA/poly(acryloyl hydroxyethyl starch)</td>
<td>Adsorption and encapsulation by solvent extraction</td>
</tr>
<tr>
<td>PLGA/ε PCL</td>
<td>Water-in-oil-in-water emulsion and evaporation</td>
</tr>
<tr>
<td>Microspheres</td>
<td>Precipitation from solution under reduced pressure</td>
</tr>
<tr>
<td>Microparticles</td>
<td>Double emulsion (w/o/w)</td>
</tr>
</tbody>
</table>

- **Release of insulin.** (Jiang et al., 2003).

- **Release of heparin.** (Jiao et al., 2002).

- **Release of progesterone and estradiol.** (Bunten et al., 1998).

- **Potential for release of water-soluble and -insoluble drugs.** (Yang et al., 2003).

- **Release of human recombinant erythropoietin.** (Morlock et al., 1997; Morlock et al., 1998).

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Encapsulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microspheres</td>
<td>Dilatification</td>
</tr>
<tr>
<td>Microparticles</td>
<td>Inverse (w/o) emulsion polymerization</td>
</tr>
<tr>
<td>Microparticles</td>
<td>Free radical emulsion polymerization</td>
</tr>
<tr>
<td>Microparticles</td>
<td>Anionic polymerization in the presence of series of cyclodextrins and derivatives</td>
</tr>
</tbody>
</table>

- **Release of clonazepam (anticonnvulsant).** (Jeong et al., 1998).

- **Release of ciprofloxin (antibiotic).** (Kriwet et al., 1998).

- **Release of peptides and other hydrophilic drugs.** (Yan and Gemeinhart, 2005).

- **Release of cisplatin.** (Duchêne et al., 1999).

- **Encapsulation of steroid-loaded cyclodextrins.**

- **Adsorption and release.**

- **Incorporation and release.**

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Incorporation and release</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polyisohexylcyanoacrylate (PIHCA)</td>
<td>Emulsion polymerization</td>
</tr>
<tr>
<td>Poly(MePEGcyanoacrylate-co-hexadecylcyanoacrylate)</td>
<td>Emulsion polymerization</td>
</tr>
</tbody>
</table>

- **Endocytosis of ampicillin and gentamicin nanoparticles for intracellular delivery.** (Henry-Michelland et al., 1987).

- **Release of tamoxifen.** (Brigger et al., 2001).
<table>
<thead>
<tr>
<th>Material</th>
<th>Type</th>
<th>Method</th>
<th>Application</th>
<th>Description</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<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>
</tr>
<tr>
<td></td>
<td></td>
<td>Dispersion polymerization</td>
<td></td>
<td>Delivery of HIV-1 Tat protein for vaccination applications</td>
<td>(Fundueanu et al., 2001; Caputo et al., 2004)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Suspension radical co-polymerization</td>
<td></td>
<td>Buformin tosylate – a classical hypoglycaemic drug</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Microparticles</td>
<td>Free-radical solution</td>
<td>Entrapment and release</td>
<td>Release of insulin</td>
<td>(Morishita et al., 2002)</td>
</tr>
<tr>
<td>Poly(methacrylic acid-g-ethylene glycol) (P(MAA-g-EG))</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>
</tr>
<tr>
<td>Poly(trimethylene carbonate)-poly(ethylene glycol)-poly (trimethylene carbonate) (PTC-PEG-PTC)</td>
<td>Nanoparticles</td>
<td>Polymerization</td>
<td>Carrier for antigen</td>
<td>Delivery of the antigen of Aspergillus fumigatus for immune system response</td>
<td>(Madan et al., 1997)</td>
</tr>
<tr>
<td>Polyvinylpyrrolidone (PVP)</td>
<td>Nanoparticles</td>
<td>Nanoprecipitation</td>
<td></td>
<td>Nifedipine (calcium antagonist) and propranolol HCl (β-blocker), for treatment of hypertension</td>
<td></td>
</tr>
<tr>
<td></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>
</tr>
<tr>
<td>Poly(diethylaminoethyl-g-ethylene glycol)</td>
<td>Microparticles</td>
<td>Reverse micelle solvent evaporation</td>
<td>Simple and double emulsion—solvent evaporation</td>
<td>Release of superoxide dismutase, Release of nitrofurantoin (antibacterial agent), Release of vancomycin, Release of fludrocortisone acetate for hormonal therapy, Release of diclofenac</td>
<td>(Dubertnet et al., 1987; Pérez et al., 2000; Gibaud et al., 2002a, 2002b; Le Ray et al., 2003; Schafflitzek et al., 2003; Youan, 2003; Gibaud et al., 2004)</td>
</tr>
<tr>
<td>ε-Polycaprolactone (ε-PCL)</td>
<td>Microparticles</td>
<td>Reverse micelle solvent evaporation</td>
<td>Simple and double emulsion—solvent evaporation</td>
<td>Simple and double emulsion—solvent evaporation</td>
<td>Incorporation and release</td>
</tr>
<tr>
<td></td>
<td>Nanoparticles</td>
<td>Nanoprecipitation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Natural polymers and blends</td>
<td>Microparticles</td>
<td>Suspension polymerization</td>
<td>N.A.</td>
<td>N.A.</td>
<td>(Abraham et al., 2002)</td>
</tr>
<tr>
<td>Polystyrene</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>
</tr>
<tr>
<td>Cytoline 2® (polyethylene and silica)</td>
<td>Microparticles</td>
<td>N.A.</td>
<td>Carrier of antigen</td>
<td>Carrier for cell culture</td>
<td>(Matzelle and Babensee, 2004)</td>
</tr>
</tbody>
</table>

Table 1. (Continued)
<table>
<thead>
<tr>
<th>Material</th>
<th>Form</th>
<th>Preparation Method</th>
<th>Encapsulation/Release Method</th>
<th>Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alginate Beads</td>
<td>Beads</td>
<td>Physical crosslinking of calcium ions to sodium alginate polymer (gelation) by needle extrusion</td>
<td>Incorporation and release</td>
<td>Release of bFGF, release of glucocorticosteroids, release of VEGF, encapsulation of cells for angiogenic factors release, magnetic affinity adsorbents for purification of enzymes, cell encapsulation of human kidney 293 cells, release of l-lactate dehydrogenase enzyme, release of model compounds, incorporation of Aeromonas hydrophila for fish oral vaccination</td>
</tr>
<tr>
<td>Alginate MicroParticles</td>
<td>Microspheres</td>
<td>Atomization and gelation using Ca(^{2+}) Microemulsion Gelation using micro-nozzle array Spray drying Spray-coagulation method</td>
<td>Carrier for cells Purification Incorporation and release</td>
<td>Delivery of several vaccines, incorporation of glucose oxidase for biosensors, delivery of ibuprofen, release of bFGF, release of NGF, encapsulation of bifidobacteria for food applications, release of antisense oligonucleotides, simultaneous incorporation of ketoprofen-loaded microspheres and rat pancreatic islets, release of model compound (albumin)</td>
</tr>
<tr>
<td>Alginate–heparin MicroParticles</td>
<td>N.A.</td>
<td>Air atomization Gelation with Ca(^{2+}) and crosslink</td>
<td>Incorporation and release Carrier for cells</td>
<td>Cell encapsulation (BHK fibroblast and C2C12 myoblast), release of BDNF, incorporation of mytomycin-C for chemothermobilization, release of steroids, release of teicoplanin, release of metoclopramide for emesis prevention, release of gentamicin, release of model agent ovalbumin, release of model drug celodipine, release of insulin for intestinal absorption, release of doxorubicin (anticancer agent), DNA carriers, release of silk peptide, release of propranolol hydrochloride (β-blocker)</td>
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<td>Alginate–poly-L-lysine Microspheres</td>
<td>Capsules</td>
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<td>Alginate–poly-L-ornithine Beads</td>
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<tr>
<td>Chitosan–poly(methyl vinyl ether-co-maleic anhydride) (CH-PVM/MA) Nano particles</td>
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<td>Template polymerization Spray drying</td>
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<td>Starch–acetate</td>
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<td>N.A.</td>
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<tr>
<td>Starch–acetate</td>
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<td>N.A.</td>
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<tr>
<td>Starch–acetate</td>
<td>Microspheres</td>
<td>N.A.</td>
<td>Incorporation and release</td>
<td>Adjuvant for oral immunization</td>
<td>(Frazzati-Gallina et al., 2001)</td>
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<tr>
<td>Material Type</td>
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<td>Processing Method</td>
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<tr>
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<tr>
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<td>Microparticles</td>
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<tr>
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<td>Self assembly</td>
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<tr>
<td>Poly(L3-hydroxybutyrate-co-3-hydroxyvalerate) (P3HBV)</td>
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<td>Poly(γ-methyl-L-glutamate)</td>
<td>Microspheres</td>
<td>Suspension–evaporation</td>
<td>Carrier for cell culture</td>
<td>Cell culture (Kato et al., 2003)</td>
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<td>Hydroxyapatite (HA)</td>
<td>Spherical granules</td>
<td>N.A.</td>
<td>Adsorption and release</td>
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<td></td>
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<tr>
<td>Ceramics</td>
<td>Granules</td>
<td>N.A.</td>
<td>Adsorption and release</td>
<td>Plasma sprayed to coat scaffolds for bioactivity induction (Weng et al., 2002)</td>
<td></td>
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<tr>
<td>Bioactive glass</td>
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<td>Potential for release of bone bioactive agents (cytochrome c as model) (Kollev et al., 2002; Matsumoto et al., 2004)</td>
<td></td>
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<tr>
<td>Coral (exoskeleton from madreporic corals)</td>
<td>Particles</td>
<td>N.A.</td>
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<td></td>
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<tr>
<td>β-Tricalcium phosphate (β-TCP)</td>
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<td>N.A.</td>
<td></td>
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<td>Silica aerogel</td>
<td>Microparticles</td>
<td>Sol–gel process using supercritical fluids</td>
<td></td>
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<tr>
<td>Si–Ca–P xerogels</td>
<td>Granules</td>
<td>Sol–gel process</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Hollow ceramic (58–72% SiO2, 28–42% Al2O3 wt%)</td>
<td>Microparticles</td>
<td>(N.A.) Coated with synthesized calcium hydroxyapatite (HA) particulate sol</td>
<td></td>
<td></td>
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<tr>
<td>Composites</td>
<td>Microparticles</td>
<td>Solvent evaporation/extraction</td>
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<tr>
<td>Biphasic calcium phosphate (BCP)/β-PCL particles</td>
<td>Microparticles</td>
<td>Dispersion polymerization</td>
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<tr>
<td>HA (coralline)–alginite</td>
<td>Microspheres</td>
<td>Encapsulation and release</td>
<td></td>
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</tr>
<tr>
<td>Polyacetic–bioactive glass (PLA/BG)</td>
<td>Microspheres</td>
<td>Solvent evaporation</td>
<td></td>
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</tr>
<tr>
<td>Starch–polyactic acid–bioactive glass (SPLA/BG 4555)</td>
<td>Microparticles</td>
<td>Solvent evaporation/extraction</td>
<td></td>
<td></td>
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</tr>
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</table>

N.A., information not available; o, oil; w, water.
The use of synthetic polymers as carriers has predominantly focused on polyhydroxyalkanoates (Ueda 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-DL-lactide-co-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 (Kirker-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).

Polymers 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 fulfil 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.

Polymers, 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 hyaluronan 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 is being actively investigated for sustained delivery of therapeautic 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 (Azevedo 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 Skjak-Braek, 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).

Polylactidone is a polyester produced as granules 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 materials, which are refractory, polycrystalline compounds, composed of ionically bonded compounds (de Groot, 1991).
1983; Bajpai and Billote, 1995). Ceramic materials, such as tricalcium phosphate (TCP), hydroxyapatite (HA) and bioactive glasses (BG) have been widely investigated for hard tissue applications (Balla et al., 1991; Schepers et al., 1991, 1993, 1998; Meenen et al., 1992; Gatti et al., 1994; Schepers and Ducheyne, 1997; Chu et al., 2002; Huygh et al., 2002; Artzi et al., 2005; Kim et al., 2005; Chu et al., 2006), for filling, support and promotion of regeneration. Their role as drug delivery devices derives from their compatibility and physical characteristics, such as non-immunogenicity and degradability. Ceramics as drug delivery systems were basically in the form of porous materials and using the well-known ceramics mentioned above. As proposed by Ducheyne and co-workers (Nicoll et al., 1997; Santos et al., 1998, 1999), sol–gel technology for the formation of silica-based xerogels, which allows the introduction of functional proteins into glass-like materials, is a very interesting strategy that couples the bioactive behaviour of these systems with drug delivery capability and the additional ability to tailor other properties. Another major advantages relate to room temperature processing without the need for solvents.

Further details on ceramic materials in bone tissue engineering can be found in the second part of this review (Silva et al., 2006).

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) topic, 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 administrated 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; Spegel et al., 2001)</td>
</tr>
<tr>
<td>Imaging</td>
<td>(Cuthbertson et al., 2003; Cavaleri et al., 2005; Huang et al., 2006; Kilbanov, 2006)</td>
</tr>
<tr>
<td>Filling of defects</td>
<td>(Schepers et al., 1991; Guicheux et al., 1997; Santos et al., 1998; Schepers et al., 1998; Falaize et al., 1999; Huygh et al., 2002; Day et al., 2004; Domingues et al., 2004; Gosain, 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; Stiertman et al., 2006)</td>
</tr>
<tr>
<td>Cell culture</td>
<td>(Malda et al., 2003b; Xu et al., 2003; Zhang et al., 2003; Liu and Wu, 2004; Yokomizo et al., 2004; Hong et al., 2005; Meler-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; Cruaud et al., 1999; Ganza-Gonzalez et al., 1999; Lam et al., 2000; Lim et al., 2000; Bigger et al., 2001; Delie et al., 2001; Han et al., 2001; Singh et al., 2001a, 2001b; van der Lubben et al., 2001; Dalpiaz et al., 2002; Demers et al., 2002; Ko et al., 2002; Morishita et al., 2002; Perez et al., 2002; Tamura et al., 2002; Yencic 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; Norton et al., 2005; Silva et al., 2005)</td>
</tr>
</tbody>
</table>
1. That the agent to be encapsulated comprises a reasonably high weight fraction (loading) of the total carrier system (e.g. >30%).
2. 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%).
3. The ability to be freeze-dried and reconstituted in solution without aggregation.
5. Small size.
6. 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 administered 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.

Figure 2. Release profile for biodegradable polymers. The first stage (1) is a burst release, caused by diffusion of the bioactive agent located closer to the surface. The second stage (2) is caused by gradual degradation of the polymer, and the third stage (3) is characterized by massive degradation (solubilization) of the material.
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 (issue) deals with the roles – played and potential – of particle-based systems in this specific subset of tissue engineering applications, bone tissue engineering.

Acknowledgements

The Portuguese Foundation for Science and Technology (FCT) is acknowledged for a PhD grant to G.A.S (SFRH/BD/4698/2001). This work was partially supported by FCT through funds from the POCTI and/or FEDER, European Union-funded STREP Project Hippocrates (NNM3-CT-2003-505758) and the European NoE EXPERTISSUES (NMP3-CT-2004-500283).

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