

Chitosan microparticles as injectable scaffolds for tissue engineering

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Abstract

The use of chitosan microparticles as injectable carriers for cell transplantation represents a promising alternative to avoid the drawbacks of the implantation of other forms of three-dimensional (3D) scaffolds seeded with cells. In this study, a 3D construct is obtained *in vitro* by combining chitosan microparticles crosslinked with genipin and goat bone marrow stromal cells (GBMCs). Cell viability and the morphology of GBMCs were evaluated after culture for 7 and 14 days. Our results show the feasibility of chitosan microparticles as potential injectable scaffolds for tissue engineering and regenerative medicine. Copyright © 2008 John Wiley & Sons, Ltd.

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Tissue engineering has progressed from the use of biomaterials, as a possible therapy for damaged tissue and organs (Langer and Vacanti 1993). Several studies have focused on seeding cells onto three-dimensional (3D) porous scaffolds in the form of hydrogels (Lee and Mooney, 2001), sponges (Tsang and Bhatia, 2004; Escobar *et al.*, 2007), fibers (Chew *et al.*, 2008), meshes (Tuzlakoglu *et al.*, 2004; Rodriguez *et al.*, 2008) or films (Costa *et al.*, 2007; da Silva, 2007) in order to guide tissue regeneration.

Recently, microspheres have also been assessed as scaffold for tissue engineering and new strategies, such as sintering (Borden *et al.*, 2002) and fusion (Malafaya *et al.*, 2005; Jaklenec *et al.*, 2008) methods, have been investigated to obtain suitable scaffolds that mimic the tissue environment for cells (Silva *et al.*, 2007; Curran *et al.*, 2005). Most of the concepts based on the use of

such scaffolds require the implantation kind of the hybrid constructs through a surgical procedure.

In this work, our goal is to combine isolated particles and cells previously incubated on their surface as a potential alternative in the context of a minimally invasive strategy, as one expects that the particles might agglomerate after implantation as a consequence of cell proliferation and extracellular matrix production playing the role of the 3D scaffold.

Chitosan has been used in the production of scaffolds for tissue engineering (Tuzlakoglu *et al.* 2004; Oliveira *et al.* 2006); simultaneously, chitosan particles have been also proposed to be used for the delivery of therapeutic molecules (Prabaharan and Mano, 2005). We will use this system to prove the above-mentioned concept. Chitosan microparticles were prepared by an emulsion crosslinking technique (Denkbas *et al.*, 1999; Al-Helw *et al.*, 1998). Briefly, 2 wt% chitosan solution in acetic acid 1% v/v was added to mineral oil, containing a surfactant (Tween 80) to form the w/o emulsion. Genipin, a molecules extracted from the fruits of *Gardenia jasminoides* Ellis was used as the crosslinking agent

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(Butler *et al.*, 2003) at different concentrations (20 or 40 mM). Emulsion was stirred overnight for 17 h and the crosslinked chitosan microparticles were recovered by centrifugation. The morphological, surface appearance and the size of crosslinked chitosan microparticles were examined by optical microscopy (Figure 1) showing dark-bluish coloured spherical particles with very smooth surface. The microsphere diameter was 70 ± 20 μm and 60 ± 10 μm for crosslinked particles with 20 and 40 mM genipin, respectively. The colour was deepened with the increase of genipin concentration and bluish colour was attributed to double bonds in the genipin crosslinking molecules.

To analyse the biological response, goat bone marrow stromal cells (GBMCs) were seeded on chitosan microparticles and cultured for 7 and 14 days. Cell viability and proliferation, as evaluated by the MTS assay (Figure 2), demonstrate that the studied chitosan provided an adequate environment for cell attachment and growth. Viability was higher for cell cultures with chitosan microparticles crosslinked with genipin solution 20 mM than 40 mM (Figure 2). The crosslinking with genipin is produced through amine bonds and therefore a higher number of free amine groups is expected to appear at the surface of the less cross-linked microsphere, which could enhance cell attachment and consequently cell viability.

Cell morphology evaluated by SEM (Figure 3) demonstrated that after 7 days of culture the cells attached and grew favourably on the microparticles, forming interparticulate cellular bridges and small cell-particle aggregates. Note that cells are in the order of magnitude or bigger than microspheres. Cells spread actively extending filopodia that adhere on a number of substrate particles. It can be said that on the one hand cell allow

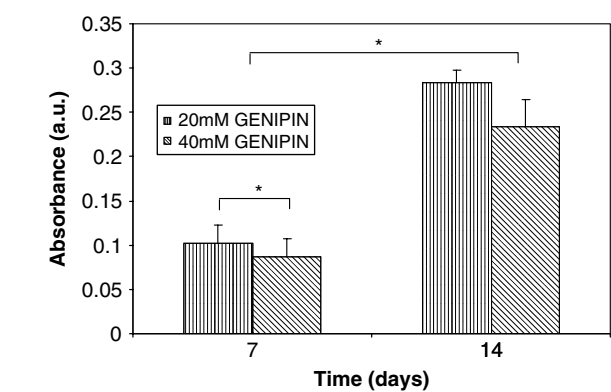


Figure 2. MTS assay performed on chitosan microparticles cultured with GBMCs. Data represented are mean \pm SD. * $p < 0.05$

microparticles to join together and on the other hand it is precisely this cell-microparticle spatial arrangement what allows chitosan to play the role of a 3D scaffold that surrounds cells. Besides, unlike conventional scaffolds, this system is highly dynamic in nature and microparticles will be displaced during cell growth, allowing the natural expansion of the tissue. Non-treated chitosan particles were used in this work for the proof of concept. However, new possibilities are open to broaden this concept. For example, combination of different kinds of particles, made of different materials or with more complex size distribution, could be used; moreover, the surface of the particles could be easily modified or a wide range of therapeutical molecules, including soluble factors, could be encapsulated in order to control cell adhesion, differentiation and proliferation.

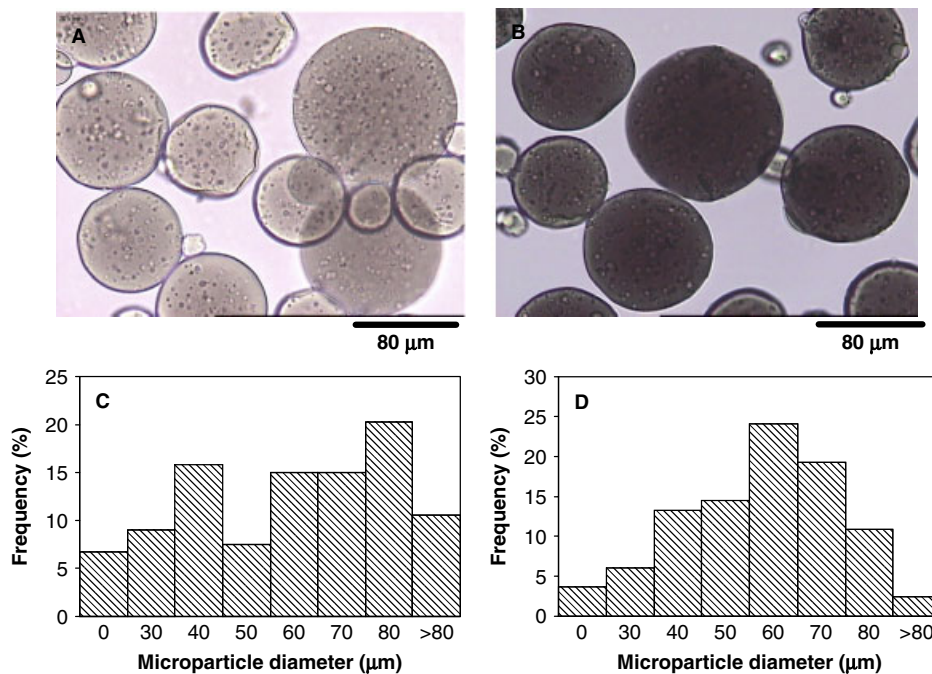


Figure 1. Morphology of chitosan microparticles cross-linked with a solution of 20 mM (A) and 40 mM (B) genipin. Their respective microparticle diameter histograms are represented in (C) and (D)

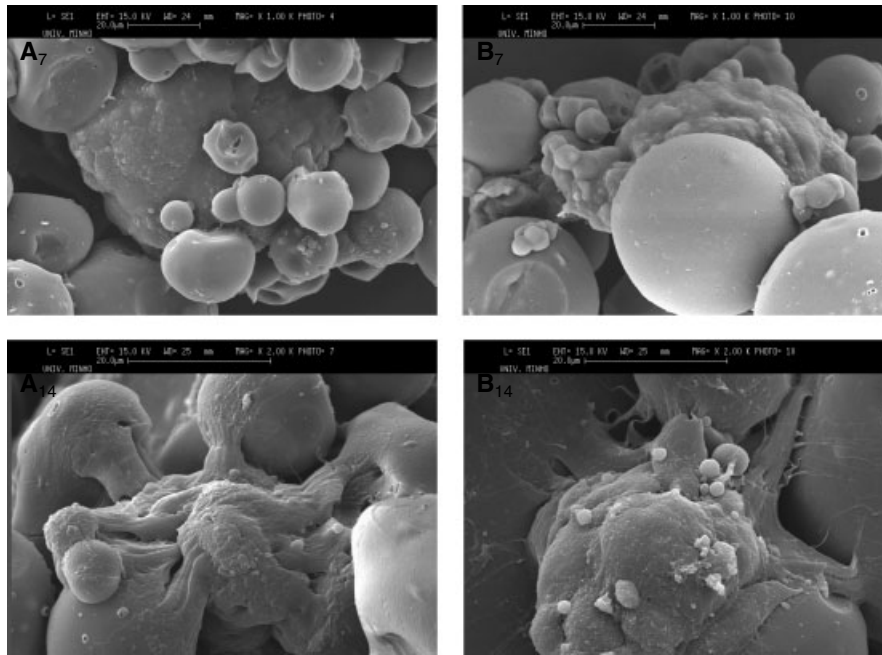


Figure 3. SEM microphotographs of GBMCs on chitosan microparticles. (A, B) Images correspond to crosslinked microparticles with 20 and 40 mm genipin, respectively; the subindices represent 7 and 14 days of culture

In conclusion, genipin-crosslinked chitosan microspheres support GBMCs adhesion and proliferation without the need of any specific surface treatment. Cells spread on the surface of the microspheres joining them to form a 3D construct in which microparticles play the role of the scaffold. Crosslinking density affects cell viability slightly but significantly. Such systems could be used in tissue-engineering strategies involving minimally invasive procedures to deliver a combination of cell, growth factors and supporting material.

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