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Ability of polyurethane foams to support cell proliferation and the differentiation of MSCs into osteoblasts

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Abstract

In bone tissue reconstruction, the use of engineered constructs created by mesenchymal stem cells (MSCs) that differentiate and proliferate into three-dimensional porous scaffolds is an appealing alternative to autologous and heterologous bone grafts. Scaffolds considered in this work are represented by polyurethane (PU) foams. Two PU foams (EC-1 and EC-2) were synthesized and characterized for morphology, mechanical properties and in vitro interaction with the osteoblast-like cell line MG63 and MSCs from human bone marrow. EC-1 and EC-2 showed similar densities (0.20 g cm^{-3}) with different morphologies: EC-1 showed a more homogeneous pore size (average $\Phi = 691$ μm) and distribution, with a 35% open porosity, whereas EC-2 evidenced a wide range of pore dimension, with an average pore size of 955 μm and a 74% open porosity. The compressive properties of the two foams were similar in the dry condition and both showed a strong decrease in the wet condition. In vitro tests showed good MG63 cell proliferation, as confirmed by the results of the MTT assay and scanning electron microscopy (SEM) observations, with a higher cell viability on EC-2 foam 7 days post-seeding. In the experiments with MSCs, SEM observations showed the presence of an inorganic phase deposition starting day 7 onto EC-1, day 14 onto EC-2. The inorganic particles (CaP) deposition was much more evident onto the pore surface of both foams at day 30, indicating good differentiation of MSCs into osteoblasts. Both PU foams therefore appeared to stimulate cell adhesion and proliferation in vitro, sustaining the MSCs' growth and differentiation into osteoblasts.

Keywords: Porous scaffold; Polyurethane; Mesenchymal stem cells; Osteoblasts differentiation; Bone tissue engineering

1. Introduction

Regenerative medicine is aimed at developing therapeutic approaches that can induce regeneration of organs or tissues affected by trauma or degenerative diseases. In particular, regeneration can be achieved by gene therapy alone [1,2], cell therapy [3] or tissue engineering [4,5], both of which can be sustained by gene transfer or drug delivery. In recent years, the efforts of numerous researchers and biotechnological industries have led to the development of new materials [6–9] and techniques [10,11] employed for bone reconstruction. Nevertheless, despite the intense research activity, the gap between in vitro studies and clinical applications is still far from being overcome.

For bone regeneration, autologous bone, demineralized heterologous bone or bone substitutes are currently used in clinical practice, but all these approaches have drawbacks. Autologous bone is rarely available, its harvesting often requires painful invasive surgery and its use is limited by the lack of donor sites. On the other hand, the transplant from a donor exposes the patient to infective pathogens and it might also be rejected. The need of, together with the lack of effective solutions for, bone tissue regeneration

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indicate the great benefit that could be achieved from alternative sources for the reconstruction of bone and tissue defects and, more specifically, from engineered constructs that can be integrated into the surrounding tissues. The use of synthetic or natural polymers for bone regeneration is extremely appealing in the clinical field, since they can be easily fabricated into three-dimensional (3-D) structures that fit the defect size well, presenting a large surface for cell adhesion and migration and a controlled porosity that allows for an adequate diffusion of nutrients and waste products.

Every scaffolding material is associated with certain strengths and limitations. Non-degradable polymers are useful for maintaining the construct shape in vivo, thus offering better resistance to structural collapse, whereas a rapid biodegradation rate may lead to the loss of stability, construct distortion and tissue loss. However, non-degradable materials will remain as a persistent foreign body after implantation, potentially eliciting a severe inflammatory reaction.

Synthetic biodegradable polymers most frequently used for tissue regeneration include poly(α-hydroxyesters), polydioxanone, polyorthoesters, polyanhydrides and some polyurethanes [12–16,43]. However, an adequate balance between in vivo degradation and tissue regeneration is not easily achievable because of a number of different variables that may occur in clinical conditions, such as the geometry of the bone defect to be filled, the different volumes of the materials required and the functional loading, which affects bone apposition and remodeling. In addition, there are concerns about toxic effects that are imputable to the local concentration of degradation products, even though they may be non-toxic per se, and the formation of non-degradable debris. Furthermore, acidic monomers or low-molecular-weight molecules leached throughout the in vivo degradation can significantly lower the local pH, therefore increasing the degradation rate of the polymer and inducing a late inflammatory response. On the other hand, natural polymers used for tissue regeneration (alginate, collagen, hyaluronic acid, gelatin, etc.) possess excellent biocompatibility, but their use is often limited by their weak mechanical properties and poor processability [17–19]. For skeletal tissues, especially bone, the lack of mechanical properties can be partially overcome by preparing composite materials with a natural or synthetic matrix and inorganic fillers (e.g. hydroxyapatite). Promising results have been obtained in recent years with biodegradable polyurethane foams, both in vitro [44–48] and in animal (rat, sheep) models [45,49], but the clinical application in humans has not yet been achieved.

Biointegration is an interesting alternative to biodegradation. It can be achieved by the use of polymeric scaffolds with a very slow degradation rate that can be designed to fulfil all the requirements of the specific application. Following this approach, scaffolds could be used effectively when there is a need to substitute great bone masses, to prevent tissue collapse and to sustain newly forming tissue. In this respect, the range of mechanical and morphological properties that can be obtained with polyurethanes (PU) is significantly larger than with commonly used medical-grade biodegradable polymers [9,20,21].

In the last 10 years we have set up a process to obtain crosslinked PU foams with a controlled range of pore size, open porosity and mechanical properties [9,22]. PU foams with different hydrophilicity [23], surface-modified by a protein coating [24] and composites [23] have been developed and investigated.

As for cells, a useful alternative to primary autologous bone cells for musculo-skeletal tissue regeneration is mesenchymal stem cells (MSCs). MSCs are multipotent cells derived from adult tissues that, with appropriate stimuli, can differentiate along different mesenchymal lineages, like osteoblasts, chondrocytes, myocytes, tenocytes, adipocytes and endothelial cells [25,26]. The potential of MSCs differentiating into osteoblasts has already been demonstrated both in vitro and in vivo [27,28]. Mesenchymal cells, when adequate culture media and appropriate scaffolds are used, are potentially able to produce an extracellular matrix typical of mature tissues. In particular, in a bone tissue engineering approach, the use of MSCs with synthetic or natural polymers with adequate chemico-physical, morphological and mechanical properties, seems to be promising in guiding tissue neo-formation after implantation in the host [28–30]. Their use seems to be advantageous in respect to differentiated osteoblasts because of their availability from different alternative sources and their capability to promote, when implanted, the regeneration of the whole complex of the bone niche, including bone and endothelial cells.

This study was aimed at investigating the proliferation of MSCs obtained from human bone marrow and their potential to differentiate into osteoblast cells when cultured on PU-foamed scaffolds. Before testing MSCs, the cytocompatibility of the PU foams was assessed with the human osteosarcoma cell line MG63.

2. Materials and methods

2.1. Polyurethane foams

2.1.1. Synthesis

Two PU foams (EC-1 and EC-2), differing in their morphological and mechanical properties, were obtained by a one-step bulk polymerization described previously [9,22], using water as the expanding agent and iron-acetylacetonate as the catalyst. Briefly, to a polyol mixture (Elasto-Coat 96827/4, EC, OH = 3.97 mmol g^{-1} polyol; Elastogran, Villanova d'Asti, Italy), water (2 wt.% polyol) and Fe-acetylacetonate (0.001 wt.% polyol) were added and mixed with a mechanical stirrer. The necessary quantity of MDI prepolymer (B141, NCO = 5.45 mmol g^{-1} isocyanate; BASF, Ludwigshafen, Germany) to obtain the stoichiomeric ratio of OH/NCO = 100/73 was then added under stirring. The reaction mixture was stirred for few