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Graded porous polyurethane foam: A potential scaffold for oro-maxillary bone regeneration

S.M. Giannitelli [a], F. Basoli [b], P. Mozetic [a], P. Piva [c], F.N. Bartuli [c], F. Luciani [c], C. Arcuri [d,e], M. Trombetta [a], A. Rainer [a,*], S. Licoccia [b]

[a] Department of Engineering, Tissue Engineering Unit, Università Campus Bio-Medico di Roma, Rome, Italy
[b] Department of Chemical Science and Technology, University of Rome "Tor Vergata", Rome, Italy
[c] University of Rome "Tor Vergata", Rome, Italy
[d] Department of Periodontics, University of Rome "Tor Vergata", Rome, Italy
[e] U.O.C.C. Odontostomatology, "S. Giovanni Calibita, Fatebenefratelli" Hospital, Rome, Italy

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ABSTRACT

Bone tissue engineering applications demand for biomaterials offering a substrate for cell adhesion, migration, and proliferation, while inferring suitable mechanical properties to the construct. In the present study, polyurethane (PU) foams were synthesized to develop a graded porous material—characterized by a dense shell and a porous core—for the treatment of oro-maxillary bone defects. Foam was synthesized *via* a one-pot reaction starting from a polyisocyanate and a biocompatible polyester diol, using water as a foaming agent. Different foaming conditions were examined, with the aim of creating a dense/porous functional graded material that would perform at the same time as an osteoconductive scaffold for bone defect regeneration and as a membrane-barrier to gingival tissue ingrowth. The obtained PU was characterized in terms of morphological and mechanical properties. Biocompatibility assessment was performed in combination with bone-marrow-derived human mesenchymal stromal cells (hBMSCs). Our findings confirm that the material is potentially suitable for guided bone regeneration applications.

1. Introduction

Bone regeneration is well known as the hardest and most delicate biological process dentistry has to face with: appropriate bone regeneration is mandatory for prosthetic rehabilitation or to restore anatomical defects. In this field, guided tissue regeneration (GTR) and guided bone regeneration (GBR) have been widely studied and validated for successful bone reconstruction.

GBR is a surgical technique promoting bone neo-formation in those anatomical sites with lack of bone amount, or with vertical, horizontal or both atrophies, restoring a bone volume suitable for implant insertion [1].

The GBR principle finds its roots in GTR of periodontal tissues first described by Nyman and Karring in 1980 [2,3]. The well-known GBR and GTR biological bases consist in creating a space next to the bone tissue, which can include a sheltered blood clot sealing it off from the gingival tissue. Sealing off is managed by semipermeable membrane-barriers able to prevent the gingival tissue ingrowth in the defect cavity. Such an environment is ready to bone regeneration, allowing the formation of a recovering pattern, which provides the migration of cells with an angiogenetic and osteogenetic potential from next-to-bone tissue medullary spaces [1].

An appropriate cavity filling material (*i.e.* graft) between the bone defect and the membrane is a crucial condition for the creation of the blood clot, and then of bone tissue. Actually, collapse of the membrane into the bone defect is the main reason of failure for GBR. Finally, the membrane holds the graft in place within the defect, avoiding its cutting off by the surrounding fibrous tissue [4].

An ideal GBR graft should meet a number of essential requirements, primarily interconnected pores of an adequate size, not only for cell migration, proliferation, and differentiation, but also for an adequate diffusion of nutrients and waste products. It should be biodegradable, and its long term *in vivo* performance should be characterized by a degradation rate compatible with the kinetics of new bone formation [5,6]. Several materials have been investigated for an application as grafts [7]. In this work, polyurethane (PU) systems have been studied as a unique material that could be used both as a membrane-barrier and a graft material in filling bone defects [8].

Thanks to their tridimensional porous structure, polyurethane (PU) foams represent one of the best candidates for the correction of bone defects [9,10]. PU foams, charged with osteoconductive, bioabsorbable

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* Corresponding author at: Università Campus Bio-Medico di Roma, via Álvaro del Portillo, 21, 00128 Rome, Italy.
E-mail address: a.rainer@unicampus.it (A. Rainer).

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inorganic salts (calcium phosphates), have been already proven as good candidates as bone graft substitutes due to their ability to stimulate cell adhesion and proliferation and to support differentiation of mesenchymal stem cells [11]. Moreover, given the highly tunable chemistry of PU systems—attributable to the alternation between hard and soft segments in the polymeric chain—scaffolds with tunable microstructure, surface roughness, and mechanical properties can be obtained [12,13].

In this work, PU foams were synthesized to develop a biphasic dense/porous material that would perform at the same time as an osteoconductive scaffold for bone defect regeneration and as a membrane-barrier for epithelial gum cells.

If performing a GTR, the polyurethane foam could be tailored into the required shape, with a smooth external side, which faces the epithelial side of the restored periodontal pocket, and an internal porous side, which would provide the colonization by the periodontal ligament stem cells. Moreover, the possibility to program the shape of the scaffold before surgery could make the technique easier and be within every surgeon's reach [14].

2. Materials and methods

2.1. Synthesis of PU foams

All reagents were purchased from Sigma Aldrich (St. Louis, MO) unless otherwise specified. In a typical reaction batch, 4.5 g of poly(ε-caprolactone) diol (PCL diol, average Mn 2000) were reacted at 70 °C with selected amounts of polymethylene polyphenyl isocyanate (PMDI; Voranate M220, Dow Chemicals) under vigorous stirring in the presence of 5.8 mg of 1,4-diazabicyclooctane (DABCO, >98%). To obtain foams at different stiffness levels, two different amounts of PMDI were tested: 13 wt.% and 19 wt.% (*vs.* total). PMDI incorporation was followed by a rapid addition of 200 μL of a 6% w/v albumin aq. solution (bovine albumin, fraction V). After vigorous stirring for 1 min to start the foaming reaction, the prepolymer was transferred in a polyethylene mold, where complete curing and hardening took place in ca. 1 h. As a result, a 3D sponge was obtained, due to CO_2 formation upon the reaction of residual isocyanate groups with water [15]. The reaction scheme is presented in Fig. 1.

Different mold typologies were used. For the preliminary characterization of PUs, open molds allowing free expansion of the foam were used, while graded porous scaffolds were fabricated using cylindrical silicone molds ($\phi = 7$ mm, $h = 30$ mm). Table 1 reports samples nomenclature as a function of process conditions.

2.2. Morphological characterization

Foam microstructure was characterized by scanning electron microscopy (SEM). Specimens were sectioned along the foaming direction, mounted on aluminium stubs using adhesive carbon tape, and coated with a conductive layer of sputtered gold (Emitech K550X, Quorum Technologies Ltd, West Sussex, UK). Samples were observed under a Field Emission SEM (FE-SEM, SUPRA™ 35, Carl Zeiss SMT, Oberkochen, Germany) operated at an accelerating voltage of 5 kV and at a working distance of 8 mm.

Pore size distribution was determined on an areal basis by image analysis on materialographic sections. Specimens were embedded in Shandon Cryomatrix (Thermo Fisher Scientific, Waltham, MA), snap frozen and cryosectioned into 30 μm slices (CM 1850 UV cryomicrotome, Leica, Nussloch, Germany). Slices were collected onto positively charged slides (Superfrost Ultra Plus, Thermo Fisher Scientific, Waltham, MA) and viewed under an inverted optical microscope (model Ti-E, Nikon, Tokyo, Japan) equipped with an Andor Neo 5.5 sCMOS camera (Andor Technologies, Belfast, UK). Image analysis was performed using NIS Element AR software (Nikon) on 5 different slices. Over 100 features were collected and measured for each group.

Total porosity of the foams was calculated by means of Archimedes' principle using a precision micro-balance (EL 204, Mettler

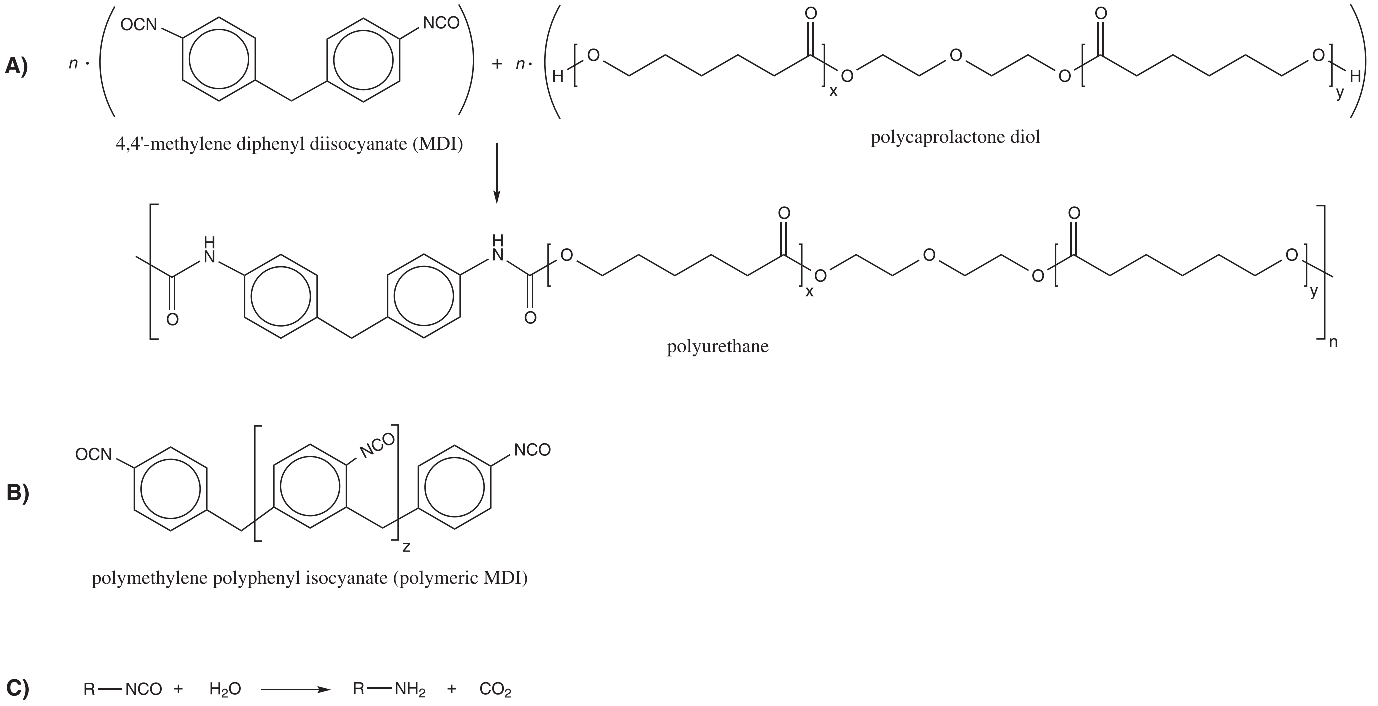

Fig. 1. A) Linear polymerization reaction between MDI and PCL diol. B) Use of polymeric MDI with functionality greater than two yields a three-dimensional cross-linked structure. C) Foaming reaction: isocyanate groups react with water giving carbon dioxide gas.