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Design and development of nanocomposite scaffolds for auricular reconstruction

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Abstract

Auricular reconstruction using sculpted autologous costal cartilage is effective, but complex and time consuming and may incur donor site sequelae and morbidity. Conventional synthetic alternatives are associated with infection and extrusion in up to about 15% of cases. We present a novel POSS-PCU nanocomposite auricular scaffold, which aims to reduce extrusion rates by mimicking the elastic modulus of human ears and by encouraging desirable cellular interactions. The fabrication, physicochemical properties (including nanoscale topography) and cellular interactions of these scaffolds were compared to Medpor®, the current synthetic standard. Our scaffold had a more similar elastic modulus (5.73 ± 0.17 MPa) to ear cartilage (5.02 ± 0.17 MPa) compared with Medpor®, which was much stiffer (140.9 ± 0.04 MPa). POSS-PCU supported fibroblast ingrowth and proliferation; significantly higher collagen production was also produced by cells on the POSS-PCU than those on Medpor®. This porous POSS-PCU nanocomposite scaffold is therefore a promising alternative biomaterial for auricular surgical reconstruction.

From the Clinical Editor: In this paper, a novel POSS-PCU nanocomposite auricular scaffold is described to reduce extrusion rates by having a much closer elastic modulus of human ears than the currently available synthetic standard. Enabling desirable cellular interactions may lead to the successful clinical application of these novel scaffolds.

Key words: Auricle; Nanocomposite; POSS-PCU; Medpor®

Ear deformity can have a significant psychological impact. It can occur as a result of trauma (67,000 patients have ear deformities caused by burns/year in the US),[1] cancer (malignant lesion of the ear account for approximately 13% of all head and neck melanomas)[2] or congenital abnormalities such as microtia and anotia (undeveloped and absence of pinna respectively, 2 per 10,000 live births in the EU).[3,4] The gold standard for total auricular reconstruction is a lengthy (up to 9 h) two-stage reconstruction procedure using sculpted autologous costal cartilage and re-arrangement of the soft tissue with complex flaps and skin grafts for coverage.[5] This approach has many complications including cartilage donor site sequelae such as scarring, and possible complications such as infection, pain and bleeding. Acquisition of the surgical techniques required to obtain aesthetically excellent results is a long and specialized process, so this skill set is not common even in plastic and reconstructive surgery.[6–8]

The use of synthetic biomaterials offers a number of advantages for auricular reconstruction. They may be personalized or mass produced in predetermined shapes and sizes, making "off-the-shelf" products possible. There is little or no donor site morbidity, and a shortened operating time and implantation would be open to many more surgeons and centers. Synthetic materials such as silicone, and porous polyethylene have been used for auricular reconstruction. At present, non-biodegradable porous high-density polyethylene (HDPE: Medpor®; Porex Surgical, Newnan, GA, USA) is the most popular synthetic material because of its stability, non-toxicity and plasticity. However extrusion and infection lead to failure rates of 14.8%.[9]

Material elastic modulus mismatch with the native tissue, inadequate cellular adhesion and delayed fibrovascular ingrowth

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into the porous implant may all contribute to failure rates.[10] The development of new implantable biomaterial for auricular reconstruction more closely matching the mechanical properties of human ears and promoting desirable cellular interactions may increase biomaterial–tissue integration and thereby prevent micro-movement and extrusion.

Recent advances in the field of material science and nanotechnology have allowed the development of biomaterials with controllable mechanical and surface properties, allowing biomaterials to more closely mimic human tissues. The use of nanotechnology, particularly the use of nanoparticles incorporated into 3D polymer matrixes, may enable even greater control over surface properties and consequently protein adsorption and tissue formation.[11] Ideal biomaterial scaffolds for auricular reconstruction should have structural stability, match the mechanical properties of the native tissue and have porosity to enable vascularization and host tissue integration.[12] Substrate stiffness also affects cellular behavior, including migration, growth and differentiation, through various mechanotransductive processes.[13]

We described the development of a non-biodegradable nanocomposite scaffold based on incorporating polyhedral oligomeric silsesquioxane (POSS) nanocage into polycarbonate-based urea–urethane (PCU, UCL-Nano™).[14,15] Tissues and organs composed of POSS-PCU have been successfully used in first-in-human applications for replacement of coronary arteries, lacrimal ducts, and the world's first synthetic trachea.[16–18] We hypothesize that this material could also be suitable for auricular reconstruction. As opposed to more complicated and costly tissue engineering approaches involving condrocytic production of ECM to provide suitable mechanical properties, our simple approach is to create a biomaterial that already matches the mechanical properties of the ear cartilage. In this approach fibrotic cell integration and preventing modular mismatch between the dermis tissue and the implant are the primary concerns to preventing extrusion rates. The aims of this study were to design and manufacture ear-shaped constructs based on POSS-PCU using two fabrication processes solvent evaporation/porogen leaching (POSS-PCUs) and phase-separation (coagulation)/porogen leaching (POSS-PCUc), to evaluate the physiochemical properties of these candidate materials to Medpor®, and third, to evaluate the biological activity of these scaffolds in terms of effects on proliferation, morphology, collagen production and migration of applied fibroblasts.

Methods

Nanocomposite polymer synthesis

POSS-PCU nanocomposite polymer was synthesised as described previously.[15] Briefly, polycarbonate polyol, 2000mwt and *trans*-cyclohexanechloroydrinisobutyl-silsesquioxane (Hybrid Plastics Inc) were placed in a 500-ml reaction flask equipped with mechanical stirrer and nitrogen inlet. The mixture was heated in order to dissolve the POSS cage into the polyol and then cooled to 70 °C. Flake 4,4′-methylenebis(phenyl isocyanate) (MDI) was added to the polyol blend and then reacted, under nitrogen, at 75 °C–85 °C for 90 min to form a pre-polymer. Dimethylacetamide (DMAC) was added slowly to the pre-polymer to form a solution; the solution was cooled to 40 °C. Chain extension of the pre-polymer was carried out by the dropwise addition of a mixture of ethylenediamine and diethylamine in DMAC to form a solution of POSS-modified polycarbonate urea–urethane in DMAC. Micro thin sheets micro thin Medpor® sheets medical grade high-density porous polyethylene (HDPE) (Porex Surgical, Newnan, GA, USA) were used as comparison.

Ear mould design

A custom-made ear-shaped 3D negative glass mould (Glossary Co., UK) was fabricated from positive 3D printing of a 3D scan obtained from the external part of the human ear, and used to manufacture a polymeric POSS-PCU auricle of appropriate size and morphology (Figure 1, *A-B*). The 3D printing or additive layer manufacturing (ALM) of auricle framework was created by a ZPrinter® system (3D Systems Corporation, USA) based on powder bed and inkjet 3D printing techniques. ALM software was used to slice the 3D computer model (standard triangulate language [STL] files format) into thinner horizontal layers of powder which is solidified by a binder. The 3D printing auricle was then fabricated layer by layer via rapid tooling (RT) process from computer sliced data to print the mould for the ear model (Figure 1, *C*). The 3D negative glass mould was then created from the 3D printing ear model and acted as a rigid cast for shaping and discharging of two different polymeric auricle POSS-PCU nanocomposites processed by solvent evaporation/porogen leaching (POSS-PCU_S) and phase-separation (coagulation)/porogen leaching (POSS-PCU_C; Figure 1, *D-E*).

Fabrication of scaffold

POSS-PCU auricular nanocomposite scaffolds were fabricated by combining porogen leaching with either solvent casting or coagulation techniques. In all procedures, sodium chloride (NaCl) was dissolved in an 18 wt% solution of POSS-PCU in DMAC containing Tween-20 surfactant. Prior to this, the porogen particles were sieved using stainless steel sieves (Fisher Scientific) in order to achieve an average particle size of 140-150 μm. The viscous slurry of NaCl/POSS-PCU was produced by dispersing and degassing of the mixture using a Thinky AER 250 mixer (Intertonics, Kidlington, UK). The weight ratio of NaCl to POSS-PCU was controlled to 3:7.

In the solvent evaporation/porogen leaching POSS-PCU_S method, the negative mould was coated with a layer of the NaCl/POSS-PCU slurry and left in the air-circulating oven at 65 °C for 4–5 h until all the solvent evaporated. This procedure was repeated four times until the polymer was adequate thickness. The ear construct was then carefully removed from the glass mould and submerged in deionized water to dissolve out the porogen to form the porous polymeric scaffold. The construct was continuously washed with frequent water changes, using pure deionized water, for a period of 72 h to ensure the complete removal of NaCl. In the phase-separation (coagulation)/porogen leaching (POSS-PCU_C) method the slurry mixture was, however, cast onto the mould and immediately immersed in