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Chemical group-dependent plasma polymerisation preferentially directs adipose stem cell differentiation towards osteogenic or chondrogenic lineages

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ABSTRACT

Human adipose derived stem cells (ADSCs) are being explored for the repair of craniofacial defects due to their multi-differentiation potential and ease of isolation and expansion. Crucial to using ADSCs for craniofacial repair is the availability of materials with appropriate biomechanical properties that can support their differentiation into bone and cartilage. We tested the hypothesis that different modifications of chemical groups on the surface of a nanocomposite polymer could increase human ADSC adhesion and selectively enhance their osteogenic and chondrogenic differentiation. We show that the COOH modification significantly promoted initial cell adhesion and proliferation over 14 days compared to NH_2 surfaces. Expression of focal adhesion kinase and vinculin was enhanced after plasma surface polymerisation at 24 h. The COOH modification significantly enhanced chondrogenic differentiation as indicated by up-regulation of aggrecan and collagen II transcripts. In contrast, NH_2 group functionalised scaffolds promoted osteogenic differentiation with significantly enhanced expression of collagen I, alkaline phosphatase and osteocalcin both at the gene and protein level. Finally, chorioallantoic membrane grafting demonstrated that both NH_2 and COOH functionalised scaffolds seeded with ADSCs were biocompatible and supported vessel ingrowth apparently to a greater degree than unmodified scaffolds. In summary, our study shows the ability to direct ADSC chondrogenic and osteogenic differentiation by deposition of different chemical groups through plasma surface polymerisation. Hence this approach could be used to selectively enhance bone or cartilage formation before implantation *in vivo* to repair skeletal defects.

Statement of Significance

Human adipose derived stem cells (hADSCs) are an exciting stem cell source for regenerative medicine due to their plentiful supply and ease of isolation. However, the optimal environmental cues to direct stem cells towards certain lineages change have to has not been identified. We have shown that by modifying the surface of the scaffold with specific chemical groups using plasma surface polymerisation techniques we can control ADSCs differentiation. This study shows that ADSCs can be differentiated towards osteogenic and chondrogenic lineages on amine (NH_2) and carboxyl (COOH) modified scaffolds respectively. Plasma polymerisation can be easily applied to other biomaterial surfaces to direct stem cell differentiation for the regeneration of bone and cartilage.

1. Introduction

Craniofacial defects caused by congenital deformities, cancer, trauma or burns remain one of the greatest challenges for plastic and reconstructive surgeons [1]. Currently, surgical options to restore bone and cartilage defects includes autologous grafts, causing donor site morbidity and are limited by the availability of suitable tissue [1]. Synthetic options available include inert materials

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such as porous polyethylene Medpor, which acts as a mechanical support for tissue ingrowth but does not allow for bone or cartilage regeneration [1]. Therefore, there is a clinical demand to create advanced materials, which can promote bone and cartilage formation [1].

Adipose derived stem cells (ADSCs) have demonstrated to be an exciting stem cell source for regenerative medicine due to their ease of isolation, high proliferative capacity and multi-differentiation potential [2,3]. In order to be able to use this readily accessible source of stem cells for repairing skeletal tissue in the clinic, optimal conditions for their differentiation and maturation are needed. One approach to restoring craniofacial defects is to create biomaterials that can guide stem cell behavior towards bone and cartilage formation. It is well known that surface chemistry can affect stem cell adhesion, proliferation and differentiation, proving to be an important parameter when considering biomaterial fabrication. Previously, to evaluate the effect of surface chemistry on stem cell behavior, self-assembled monolayers have been utilised [2,4,5]. Curran et al. demonstrated that clean silane modified surfaces with NH_2 surfaces promoted osteogenesis but not chondrogenesis of mesenchymal stem cells (MSCs) [4]. However, self assembled monolayers (SAMs) are limited to evaluating gold and glass substrates.

Plasma surface modification is an effective and economical technique, which can be used to modify the surface chemistry of biomaterials with various shapes and sizes and then study cellular interactions [6,7]. The plasma process results in a physical and chemical modification of the surface of the biomaterial, while its bulk properties remain unchanged [7]. Plasma polymerisation allows the introduction of a wide range of surface chemistries and forms a layer of adherent functional groups on the biomaterial surface [7]. The process involves activating the surface with gases such as oxygen, nitrogen or argon and then depositing the desired functional groups onto the material surface [7].

We have previously demonstrated that our nanocomposite scaffold, which encompasses polyhedral oligomeric silsesquioxane (POSS) nanoparticles within a polyurethane backbone, can support the ADSC adhesion and growth *in vitro* [3]. We have previously shown that we can modify POSS-PCU with NH_2 and COOH functionalisation using plasma polymerisation [8]. Allylamine and acrylic acid were used to deposit —NH_2 and —COOH groups, respectively, on the nanocomposite scaffolds [8]. We also have some initial data that allylamine modification may increase osteogenic differentiation of ADSCs [9]. Here we tested the hypothesis that different modifications of the chemical groups on the surface of the nanocomposite polymer could increase adhesion of ADSCs to the nanocomposite scaffold and selectively enhance their differentiation towards bone or cartilage. We show here that by varying the chemical functionality on the surface of the nanocomposite scaffolds we can indeed enhance adhesion as well as preferentially stimulate human ADSC differentiation capability towards the chondrogenic or osteogenic lineage. These results demonstrate that plasma polymerisation of biomaterials can be a useful tool for improving the skeletal differentiation of ADSCs.

2. Materials and methods

All reagents and tissue culture plastic were from Sigma Alrich (UK) unless otherwise specified.

2.1. POSS-PCU nanocomposite synthesis and 3-dimensional (3D) scaffold fabrication

The nanocomposite polymer, POSS-PCU, was synthesised, as described previously [8]. Briefly, polycarbonate polyol (2000 mwt) and trans-cyclohexanechloroydrinisobutyl-silses-106 quioxane (Hybrid Plastics Inc) was placed into a 500 ml flask containing a mechanical stirrer and nitrogen inlet. The POSS cage was dissolved into the polyol solution using heat followed by cooling to 70 °C. At a temperature of 75–85 °C for 90 min flake 4,4′-methylenebis(phenyl 109 isocyanate) (MDI) was added to the polyol blend mixture to form a pre-polymer. To create a solution dimethylacetamide (DMAC) was then added slowly to the pre-polymer. Following cooling to 40 °C chain extension was then carried out by the addition of ethylenediamine and diethylamine in DMAC in a drop wise manner. This process created a POSS-modified polycarbonate urea-urethane in DMAC solution.

The POSS-PCU polymer was fabricated as a 3D scaffold using a coagulation technique. Firstly, sodium chloride (NaCl) was dissolved in 18% weight solution of POSS-PCU in DMAC containing Tween-20 surfactant. Stainless steel sieves (Fisher Scientific, Loughborough UK) were used to obtain a NaCl mixture of 200–250 μm size. The final solution was then dispersed and degassed in a Thinky AER 250 mixer (Intertronics, Kidlington, UK). A 1:1 wt ratio of NaCl to POSS-PCU was used in all experiments. The polymer mixture was then spread evenly onto circular stainless steel moulds and directly placed into deionised water for initially 30 h. Following this period, frequent water changes were carried out to dissolve out to remove the NaCl porogen particles and DMAC from the polymer solution for 7 days to create a porous scaffold. Then 8 cm × 8 cm circular polymer sheets with 700–800 μm thickness were manufactured. For cell culture analysis the circular sheets of polymer were cut into 16 mm diameter discs to be used in 24-well plates, using a steel manual shape cutter. Prior to cell seeding polymer discs were briefly sterilized using 70% ethanol and washed three times with sterile phosphate buffer (PBS) as previously reported [8]. They were then incubated in Dulbecco's Modified Eagle's Medium/Nutrient Mixture F-12 Ham (DMEM/F12) supplemented with 10% fetal bovine serum (FBS) and 1% antibiotic solution (penicillin) for 24 h prior to cell seeding.

2.2. POSS-PCU 3D scaffold surface modification with plasma

Plasma surface modification was carried out by using low (radio frequency) plasma generator operating at 40 kHz at 100 W. Scaffolds to be treated were placed in a 24 well plate [8]. Plasma modification was performed as a 2-stage procedure, surface activation and plasma polymerisation. Surface activation was achieved by exposing samples to oxygen plasma for 5 min, at 40 kHz. With gas flow rate of 0.4 mbar. Plasma polymerisation was carried out by introducing either allylamine or acrylic acid monomers (Sigma Aldrich, UK) at 0.4 mbar pressure for further 5 min at 100 W to produce NH2 and COOH scaffolds, respectively. Samples were immediately stored in a desiccator under vacuum until further use.

2.3. Bicinchoninic acid (BCA) assay protein quantification assay

Total serum protein adsorption on unmodified and modified samples was determined by using BCA assay (ThermoFisher Scientific) as described previously (n = 6) [8]. Briefly, scaffolds were incubated with complete growth medium at 37 °C for 1 h. The scaffolds were washed three times with phosphate buffer saline (PBS, pH = 7.4) before adding BCA reagent to each well and incubated at 37 °C. The absorbance was measured at 562 nm (Fluoroskan Ascent FL, Thermo Labsystems, UK). Scaffolds incubated in serum free medium were used as a control (n = 6).

2.4. Specific protein adsorption to plasma modified scaffolds: fibronectin and vitronectin

Specific protein adsorption to plasma-modified scaffolds was performed according to Seo et al. [10]. Each POSS-PCU scaffold