Testing of a 3D Printed, Nanostructured Osteochondral Implant for Knee Repair in a Small Animal Model


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ABSTRACT

Osteochondral lesions of the knee are difficult injuries to treat [1, 2]. Despite improvements in the diagnosis of these lesions, optimal treatment remains elusive, likely as a result of the complex interactions between host factors and lesions specific factors. Lesions with disrupted cartilage that are unstable are especially difficult to treat in younger and more active patients, with current treatment methods leading to mixed results overtime [3]. New and novel materials used to treat joint injury in these populations need to be compatible with industrial scale quality and economies of scale in order to serve as commercially viable implantable devices. We investigated the feasibility of using three-dimensional biologically inspired implants, manufactured using novel 3-dimensional printing techniques and synthetic bio-nanomaterials for treatment of osteochondral defects in a rodent model.

Keywords: 3D printing, stem cells, nanomaterial, orthopedics

1 INTRODUCTION

Repair of full thickness cartilage and early osteochondral lesions, due to the difficult clinical challenges they pose, has attracted many researchers over the years [4]. Osteochondral bone scaffolds serve the purpose of providing a complex structure, often comprised of different patterns or even different materials which attempt to stimulate bone and cartilage tissue in-growth and development similar to the native articular tissue [5]. The osteochondral region is anatomically characterized by the subchondral bone, a highly calcified and well aligned transitional region and the articular cartilage. Each layer has very specific protein, mineral and other material components, as well as highly organized and varying arrangements and alignments of the materials and cells [2, 6]. Recreating the material composition and structure of this region is essential to compiling artificial regenerative bone tissue [7].

In the field there has already been some advancement. Sharma et al used an injectable thermosetting hydrogel based technology developed in a goat model to conduct a critical sized cartilage repair study in humans [8]. The study focused on a biodegradable material, paired with microfracture surgery, aimed at full cartilage tissue regeneration. Initial results were good but device filed in a larger study, likely due to poor mechanical support of the lesion prior to full cartilage healing, something which is essential [9]. Evaluation of 3D printed solutions are in a much more early stage, but Zhang et al investigated subchondral bone structural parameters and cartilage growth for large osteochondral defect repair [10]. Osteochondral composites were fabricated using 3D printing and implanted into critical sized defects in rabbits. While these results were promising, they lacked mechanical functionality and failed to adequately vascularize.

To that end this project utilized a series of different micro-geometric porous structures which can be 3D printed. The structures have been designed to promote efficient native cartilage, bone and vascular in-growth in appropriate and specified locations, and to have favorable mechanical and blood flow properties. This allows the synthetic printed implant to both support mechanical loading similar to native bone and cartilage, and to promote fast and efficient fluid perfusion, namely of arterial blood, throughout the implant. This is especially important for initial induction of progenitor cells and early vascular formation deep within the implant microarchitecture. Current work in 3D printing artificial vascularized bone [11, 12] has begun to set a foundation for clinical treatment, but has largely only been effective for generating new vasculature alone. The use of one multi-functional material in a bi or tri-phasic 3D printed design may also be a highly desirable approach, as the cartilage and bone layers of biomaterial implants composed of dissimilar materials often dissociate in situ [13].

Another significant feature of this project is the use and evaluation of a new class of nano-porous thermoplastic polyurethanes (TPU), and their optimization for 3D printing and biological use. The TPU material is highly highly elastic, yet strong, and acquires an aligned nonporous topography [14]. This in turn combined with the 3D printed
microporous structure to create a hierarchical micro to nano porosity which has been shown to be extremely advantageous for stem cell recruitment, growth and differentiation [15]. These materials also have physical characteristics, compressive and elastic properties very similar to native cartilage at the osteochondral region, while also accelerating the formation of new bone and vascular networks [16].

Employing performance enhancing 3D structures and biologically active and analogous materials is extremely significant because a thoughtful combination can form a transitional structure which looks like osteochondral tissue, replaces the function of the lost cartilage and causes new vascularized subchondral bone to form in the implant, anchoring it permanently in place and repairing the articular surface. Our innovative use of new material and 3D structures would allow for the realization of an implant which when used on a patient could provide a quick yet complete return to mobility, while simultaneously repairing the defect long term.

2 MATERIAL AND METHODS

Experiments conducted in the project were designed to build upon previous work by Castro et al, exploring the bioactivity of 3D printed TPU scaffolds for bone tissue engineering [17]. Methods for the processing, design and printing were used, and the work presented here sought to translate initial in vitro study into a more clinically relevant in vitro and in vivo model, to evaluate complex tissue repair. A flowchart of the experimental design can be seen in Figure 1.

2.1 Scaffold Fabrication

Scaffolds were printed on both an Envision-TEC Bioplotter, as well as a tabletop Solidoodle fused deposition modeling (FDM) machine. For in vitro experimentation, scaffolds were designed in Rhinoscerous (Rhino) as 5 mm diameter disks with a 0.5 mm thickness. In vivo samples were also designed in Rhino as 2 mm diameter, 3 mm high cylinders. For all samples, scaffolds had a 250 µm feature size and layer thickness, with a 150 µm pore width and/or channel diameter. All models were exported as STL files and processed into GCODE files using Sli3er, or using Envision-TEC’s proprietary software. Three main scaffold groups were printed for the in vivo study, a positive control of polyethylene glycol (PEG) and polyethylene diacrilate (PEGDA), an experimental composite of PEG, PEGDA, nano hydroxyapatite (nHA) and polycaprolactone (PCL) and the TPU. The positive control and the PEG-PEGDA-nHA-PCL composite were prepared with a UV initiated photocrosslinker as described in our previous paper [18]. The control was cast in a petri dish as a 3 mm deep layer and exposed to high intensity UV light for 15 minutes. The composite was printed on the Envision-TEC using a UV curing tool and protocol. TPU scaffold for both experiments were printed on the Solidoodle tabletop printer.

2.2 Surgical Implantation

An osteochondral defect was created in the trochlear groove of the left knee of 6-week old female Sprague-Dawley rats. A lateral parapatellar approach to the knee was used and the defect was created with a 2.5 mm Kirschner wire. Four experimental groups included a blank control, a solid hydrogel implant and the two experimental implants. All samples were sterilized using a Sterrad XT low temperature plasma sterilizer located at Children’s National Medical Center. The osteochondral defect was created and then immediately repaired by press-fitting the experimental implant into the defect. At 1 and 3 months samples were harvested and analysed. The surgical procedure was
successful in all animals and all subjects survived to the follow-up time point.

2.3 In vitro and in vivo testing testing

For in vivo testing, samples harvested from rats were processed in Fromacal to remove calcium from the tissue. Samples were then embedded in parafin, sectioned on a microtome sectioning machine, and stained for hematoxylin and eosin. Samples were then viewed on a light microscope and photographed using a Nikon digital dual feed camera mounted on the microscope.

In vivo testing was performed using mesenchymal stem cells (MSCs) expanded and cryopreserved by John Fisher’s lab at University of Maryland and human umbilical vein endothelial cells (HUVECs) purchased from Thermo Fisher Scientific. Two groups were compared, TPU samples cultured with MSCs and TPU samples co-cultured with MSCs and HUVECs, for 5 and 10 days. TPU samples were sterilized by exposure to UV light for 30 minutes and then washing with 70% ethanol. Samples were pre-wetted for 24 hours in a mixture of 1 to 1 complete cell media (CCM) and Medium 200 containing 10% low serum growth supplement (LSGS). TPU samples were then seeded with 100,000 MSCs or 50,000 MSCs and 50,000 HUVECs, and cultured in the same mix of CCM and LSGS. Samples were then taken at the end point, fixed in 10% formalin, and treated in 1% Triton-X to increase permeability. Samples were then stained with primary and secondary antibodies to label osteopontin and von Willebrand factor, markers for bone and vascular development, as described [19]. Samples were finally imaged on an Olympus confocal microscope. Osteopontin was labeled with Texas-Red and von willebrand factor with Alexafluor 488.

3 RESULTS AND DISCUSSION

Previous work demonstrated very strongly that 3D printed TPU scaffolds in particular would yield both dense 3D calcified ECM [17]. However, vascularized bone tissue formation is critical for the successful grafting and integration of an orthopedic implant. Even in the case of a cartilage-focused replacement such as ours, the new
cartilage and the replacement need to graft adequately to the subchondral bone. In vitro study performed to evaluate vascularized bone formation (Figure 2) showed that, especially in a co-cultured environment, our 3D printed TPU scaffolds were capable of generating dense and well vascularized bone tissue. These results show that in a more biomimetic co-cultured environment, the ideal scaffolds from new bone and generate neovascular tissue, thus this behavior can be expected in situ.

Histologic analysis (Figure 3) demonstrated some bone remodeling and mostly formation of trabecular bone and marrow in the control, and in the PEG group. The PCL based experimental implant showed the formation of extensive fibrosis and scar tissue around the implant, with some bone remodeling. TPU based material showed the formation of fully remodeled bone around the implant, and some bone invading the implant microstructure. In an exciting development, a peristome like membrane formed over the articulate surface of the implant. At 3 months this yielded the formation of new fibrocartilage fusing the implant to existing articulate cartilage. It has been shown here that an experimental highly nanoporous TPU material can effectively be 3D printed into a complex microstructure implant for the repair of osteochondral lesions, accelerating bone growth with no fibrosis, as compared to controls and other experimental designs. The implant also showed signs of encouraging vascular and cartilage formation. Based on this data, a strong hypothesis can be put forth that the use of nanoporous and elastic TPU along with highly interconnected 3D printed structures there is great potential for a scaled up implant which could quickly graft to bone and even fuse to the healthy cartilage in a human patient’s defect.

REFERENCES


