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A photopolymerized composite hydrogel and surgical implanting tool for a nucleus pulposus replacement

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ABSTRACT

Nucleus pulposus replacements have been subjected to highly controversial discussions over the last 40 years. Their use has not yet resulted in a positive outcome to treat herniated disc or degenerated disc disease. The main reason is that not a single implant or tissue replacement was able to withstand the loads within an intervertebral disc. Here, we report on the development of a photo-polymerizable poly(ethylene glycol)dimethacrylate nano-fibrillated cellulose composite hydrogel which was tuned according to native tissue properties. Using a customized minimally-invasive medical device to inject and photopolymerize the hydrogel *insitu*, samples were implanted through an incision of 1 mm into an intervertebral disc of a bovine organ model to evaluate their long-term performance. When implanted into the bovine disc model, the composite hydrogel implant was able to significantly re-establish disc height after surgery ($p < 0.0025$). The height was maintained after 0.5 million loading cycles ($p < 0.025$). The mechanical resistance of the novel composite hydrogel material combined with the minimally invasive implantation procedure into a bovine disc resulted in a promising functional orthopedic implant for the replacement of the nucleus pulposus.

1. Introduction

The impact of low back pain on society is tremendous [1]. The economic burden was estimated to be up to 355 $ per capita and annum for direct costs and 507 $ for indirect costs [2] which results in a total of 200 billion per annum for a country such as the United States of America [3]. In its early stage, low back pain with or without sciatica is addressed with conservative treatments such as physical therapy or medication [4,5]. In a later stage, decompression surgery might be warranted [6] and in the case of disc herniation a more invasive procedure such as discectomy is undertaken [7]. For persisting pain due to disc degeneration fusion of one or several spinal segments might become necessary [8]. The reasons for more severe surgery are mainly disc protrusion and herniation, degenerative disc disease [9] and spondylolisthesis [10]. For all these conditions, the range of motion of the spinal segment increases which leads to a segment instability and pain [11].

The ideal solution is a stabilization of the joint by reinforcing the degenerated or missing tissue of the nucleus pulposus (NP) [12] which is the core of the intervertebral disc (IVD). However, up to date, all implantation attempts with a NP replacement material have failed due to extrusion, expulsion or subsidence of the implanted material [13,14]. The required material properties have been subject of controversial discussions over the last two decades. The current, general consensus is that more mechanically resistant materials need to be developed [15–17]. What is clear, is that a material needs to be implanted in a minimally invasive manner to

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avoid damaging existing tissue [15]. It also needs to re-establish disc height without destroying the endplate [12,16] and should not extrude when the spinal segment is cyclically loaded [13,18]. We propose an implant solution based on a photopolymerizable material. The low viscosity of the material is ideal for injecting it through a small capillary and for flowing into tissue interstices. The capillary also guides the curing light, thus providing an original solution for minimal intrusion and optimal control.

Photopolymerized implants first appeared more than 40 years ago in dentistry [19] where the initiation of the photopolymerization reaction by light was a significant advantage in terms of control and integration into enamel. Recent advances in water-based and biocompatible materials which allows photopolymerization of volumes of several cm^3, open a new avenue for *in situ* photopolymerized implants for orthopedics [20], oncology [21] and ophthalmology [22]. Especially, photopolymerized hydrogels provide promising solutions for tissue replacements [23]. However, despite the large body of work in photopolymerized hydrogels, none of them to our knowledge, has the performance required for a NP replacement, and there are no devices available for controlled, minimally invasive placement of photopolymerized hydrogels.

Hydrogels lack structural strength, and thus are not adapted to cases of high mechanical stress, such as in the IVD [24]. Conventional methods to improve the hydrogel stiffness, such as increasing the cross-linker density, usually result in a loss of strength and water content [25–27]. Different hydrogel designs have been proposed to tackle this issue [25,28] such as slip-link hydrogels [29] and double network hydrogels [30]. Although these methods result in significant improvement of stiffness and toughness, their long and/or sequential preparation makes *in situ* curing impractical. The use of a composite hydrogel is an alternative approach. Composite hydrogels combine the high stiffness and strength while preserving the one-step hydrogel preparation. The composite material retains the short curing time making the composite material suitable for *in situ* insertion.

Minimally invasive photopolymerization has been achieved *in situ* by transdermal illumination [31] and irradiation through the walls of blood vessels [32]. To achieve the photocuring deeper within tissue, methods and devices need to be developed.

Photopolymerized poly(ethylene glycol)dimethacrylate (PEGDMA) has been widely investigated for biomedical applications such as cell encapsulation [33], tissue engineering [34] and drug delivery [35]. PEGDMA is highly hydrophilic and the resulting hydrogel properties are tunable by changing the polymer's molecular weight and water content. Cellulose fibers showed to be a promising composite material for the reinforcement of the polymeric matrices [36,37]. The use of cellulose fibers to reinforce the hydrogel matrix is advantageous because cellulose is biocompatible and its addition only slightly influences the equilibrium water content [38]. Recently, in a separate study [39], the authors have shown that the hydrogel properties such as precursor viscosity, curing kinematics, swelling ratios and mechanical characteristics (elastic modulus, fracture strain/stress, energy dissipation) can be tailored by changing the concentration of nano-fibrillated cellulose (NFC) and the PEGDM molecular weight. Furthermore it has been observed that nano-fibrillated cellulose fibers also have a positive impact onto the bio-optical scattering properties of a hydrogel which results in more efficient and homogenous curing during light illumination.

In this study, relative concentration of NFC and molecular weight of PEGDMA were tuned in the composite hydrogel in order to match closely the properties of the NP native tissue. We further hypothesize that by injecting the liquid precursor and activating it through photopolymerization, the tissue integration of the implanted material is strongly enhanced.

2. Materials and methods

A hydrogel was first tailored to match the properties of native NP tissue in terms of elasticity and water content. A selected hydrogel was then evaluated against native bovine NP tissue during specifically designed functional tests: 1) confined compression was done to avoid subsidence into the IVD endplate, 2) the hydrogel's swelling pressure was tested to be able to re-establish disc height and 3) an extrusion test was performed to evaluate the hydrogel's resistance to extrusion or expulsion. Following material testing, a surgical injection, illumination and monitoring device was developed and applied to implant the composite hydrogel into an *ex vivo* bovine IVD organ culture model.

2.1. Sample preparation

2.1.1. PEGDMA synthesis

PEGDMA was synthesized according to the description by Lin-Gibson et al. [40]. Poly(ethylene glycol) with molecular weights of 6 and 20 kDa and triethanolamine (99%) were purchased from Sigma Aldrich, Buchs, Switzerland. Poly(ethylene glycol) was dried by the aid of dean-stark distillation. Extra dry dichloromethane (99.8%) and diethyl ether (99.5%, extra dry over molecular sieve) were purchased from Acros, Basel, Switzerland. Dried poly(-ethylene glycol) (20 g) was dissolved in 60 ml dichloromethane. Methacrylic anhydride (303 mg) and triethanolamine (462 mg) were added to the solution and the methacrylation was carried out under dry argon flow. After five days, the solution was precipitated in diethyl ether, filtered and dried overnight in vacuum at room temperature. The H NMR spectrum revealed a 74% and 90% degree of methacrylation for the PEGDMA 6 and 20 kDa, respectively.

2.1.2. NFC preparation

Cellulose pulp (bleached softwood pulp, elemental chlorine free) with a residual chlorine content of 0.4 wt% was purchased from Zellstoff Stendal, Arneburg, Germany. Cellulose pulp was fibrillated with a high-shear homogenizer by pumping the suspension through two consecutive chambers with diameters of 400 and 200 μm (*i.e.*, H230Z $_{400\ \mu m}$ and H30Z $_{200\ \mu m}$, respectively) for 12 passes. The resulting NFC suspension was concentrated with the aid of centrifugation (5′000 rpm, 25 °C, three times during 15 min). According to cryo-SEM images the NFCs diameter was in the range of 2–100 nm and their length in the range of a few micrometers.

2.1.3. Composite hydrogel preparation

Phosphate buffered saline (PBS, pH 7.4) was purchased from Gibco, Basel, Switzerland and 4-(2-hydro- xyethoxy) phenyl-(2-hydroxy-2-propyl) ketone (Irgacure 2959) was purchased from BASF, Basel, Switzerland. PEGDMA powder (10 wt%) and NFC (0.5 vol%) were added to PBS. The PEGDMA was dissolved by keeping the solution in 37 °C water bath for 15 min and the photoinitiator Irgacure 2959 (0.1 wt%) was added to the suspension. The suspension was then homogenized by ultra turex (IKA T25 digital, SN 25 10G, Staufen, Germany) for 20 min in a dark chamber. The homogenized suspension was degassed at a pressure of 20 mbar.

In order to characterize the hydrogel properties, the precursors were cast in plastic molds with a diameter of 8 mm and height of 4 mm, covered by microscope slides and illuminated by monochromatic 365 nm ultra violet lamp (AxonLab, Baden, Switzerland) with an intensity of 5 $mWcm^{-2}$ during 30 min.

2.1.4. Sample sterilization

The photoinitiator solution and the PEGDMA dissolved in PBS were passed through the 0.22 μm filter (Millex®GS, Millipore Corporation, Bedford, MA). NFC were autoclaved and the NFC solution