In-situ photopolymerized and monitored implants: successful application to an intervertebral disc replacement

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ABSTRACT

Photopolymerization is a common method to harden materials initially in a liquid state. A surgeon can directly trigger the solidification of a dental implant or a bone or tissue filler by using ultra-violet light. Traditionally, photopolymerization has been used mainly in dentistry. Over the last decade advances in material development including a wide range of biocompatible gel- and cement-systems open up a new avenue for in-situ photopolymerization.

We designed a miniaturized light probe where a photoactive material can be 1) mixed, pressurized and injected 2) photopolymerized or photoactivated and 3) monitored during the chemical reaction. The device enables surgeries to be conducted through a hole smaller than 500 μm in diameter.

Using a combination of Raman and fluorescence spectroscopy, the current state of the photopolymerization was inferred and monitored in real time within an in-vitro tissue model. It was also possible to determine roughly the position of the probe within the tissue cavity by analysing the fluorescence signal. Using the technique hydrogels were successfully implanted into a bovine intervertebral disc model. Mechanical tests could not obstruct the functionality of the implant. Finally, the device was also used for other application such as the implantation of a hydrogel into an aneurysm tissue cavity which will be presented at the conference.

Keywords: Polymerized medical implant, light scattering, cross-linking, injectable hydrogel, in situ photopolymerization, intervertebral disc regeneration, nucleus pulpous replacement, fluorescence monitoring

1. INTRODUCTION

Lower-back pain is experienced by 70 to 85% of the world's population once in their life[1]. The resulting cost in OECD countries is estimated to be up to $100 billion per year. The current surgical options for spinal disorders (e.g. intervertebral disc degeneration, general spine degeneration[2]) are not very effective[3]. If conservative treatments fail, the gold standard remains spinal fusion after discectomy[4,5]. Although temporarily the pain is alleviated in the short term, problems are often shifted to adjacent vertebral segments in the long run.[6] Therefore, more physiological solutions are highly warranted, i.e. minimally invasive while re-establishing pre-operation disc-height and joint motion. A considerable amount of effort is directed at replacing the jelly core (the Nucleus Pulpous) of the intervertebral disc (IVD) by a synthetic mechanical implant. A promising replacement option are photopolymerizable hydrogels[7] because their mechanical properties depend on their cross-linking density, polymer composition, degree of swelling[8] and polymerization conditions[9,10]. By understanding and controlling polymerization patterns, local material properties can be

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engineered (elastic modulus, swelling ratio e.g.), to match the set of requirements for the implant. However, during implantation it is not possible to control the photopolymerization actively. Therefore *in-situ* online monitoring methods should be developed to track the actual photopolymerization state during illumination.

The core of this work has been published recently in two SPIE Biomedical optics paper[11,12]. In this article, we further describe certain technical aspects regarding the optical device development. Moreover we report on essential biomedical aspects required for the translation into clinics such as part of the biocompatibility testing of the procedure and the *ex-vivo* implantation and mechanical testing of the photopolymerized implants

2. *IN-SITU* PHOTOPOLYMERIAZTION

During *in-situ* photopolymerization a liquid monomer is activated by light illumination. It hardens and froms a solid implant. The challenge of *in-situ* photopolymerization is the light distribution within the tissue. On one hand an illumination probe is required to be small and minimally invasive to reduce mechanical tissue damage during insertion and on the other hand space is required for optimal light distribution within the tissue cavity. Therefore, different fiber probes were built. In Fig. 1a) a diffusor-based probe distributes light radially. It can be placed within a tissue cavity and the implant will photopolymerize gradually surrounding the fiber. Fig. 1b) presents a combination between light- and injection-probe: an injection channel is surrounded by a fiber bundle. At the tip of the probe a lens with a hole in the middle is placed. The liquid polymer is ejected directly through the center of the lens. At the same time the light for photo-activation is focused and will induce photopolymerization at the focal length of the lens. Fig. 1c) shows a balloon probe where the liquid polymer is injected on the side. An optical light guide delivers light into the middle of the diffusing balloon. Thus, by deflating the balloon gradually during photopolymerization an implant can be formed layer by layer. Of the three proposed procedures the diffusor tip was selected because it proved to be the least invasive solution to replace intervertebral disc tissue.

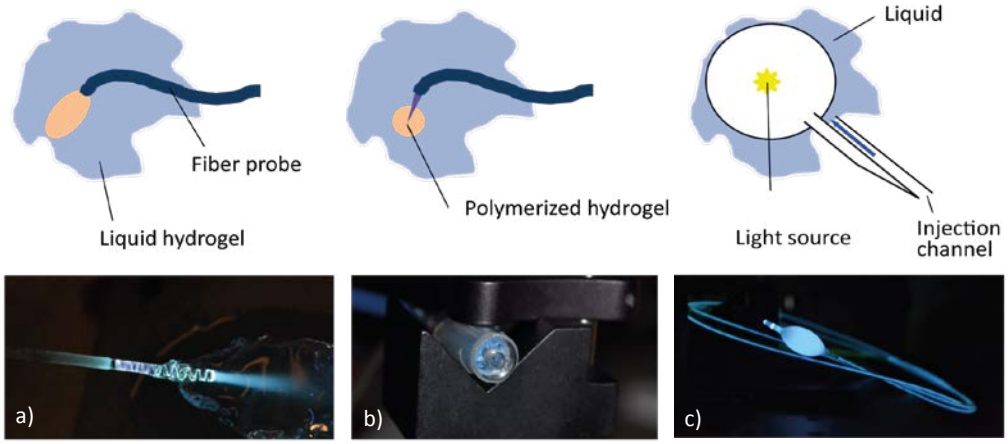

Figure 1 Three types of probe tips designed for in-situ photopolymerization: a) probe with diffusor tip, b) tip with concentric injection channel in the center of a lens surrounded by a bundle of fibers and c) balloon tip where a balloon with a light guide inside is inflated and a material may be photopolymerized layer by layer when deflating the balloon.

3. IMPLANTATION USING REACTION MONITORING BASED ON RAMAN AND FLUORESCENCE SPECTROSCOPY

To further reduce the invasiveness of the procedure the diffusor tip was replace with an untreated optical fiber and the lipid scattering particles were added to the injected liquid monomer, thus turning the material to be implanted instead of the fiber into the diffusion optical element. This addition of scattering particles required the development of a Monte Carlo model (recently published[11] in SPIE's biomedical optics journal) to predict the light distribution within the implant. Based on these simulations lipid concentrations were calculated. However these simulations also showed that

the photopolymerization of an implant requires a feedback mechanism. Therefore, a monitoring device was built (also recently published[12] in SPIE's biomedical optics). In summary two beams are used: the first to photo-activate the material injected into the tissue cavity (fig. 2a) and the second to monitor the injected material before, during and after the photopolymerization, but also to give feedback on the position of the probe (fig. 2b). It was shown that the fluorescence signal developed by the material varies over time depending on the photopolymerization state of the material. Thus, mechanical and optical properties could be correlated in real time during the photopolymerization. In fig. 2c) two photopolymerized samples are illustrated (with and without scattering particles). The addition of the scattering particles significantly changes the final form and size of the implant. The used polymer is poly(ethylene glycol)dimetacrylate with a chain length of 20 kDa specifically developed for this application. Also an injection device was developed (fig. 2d) which allowed the illumination of the photopolymer while pressurizing the injected material up to several MPa. The combination of these different steps allowed to successfully photopolymerize and implant within an intervertebral disc tissue cavity (fig. 2e).

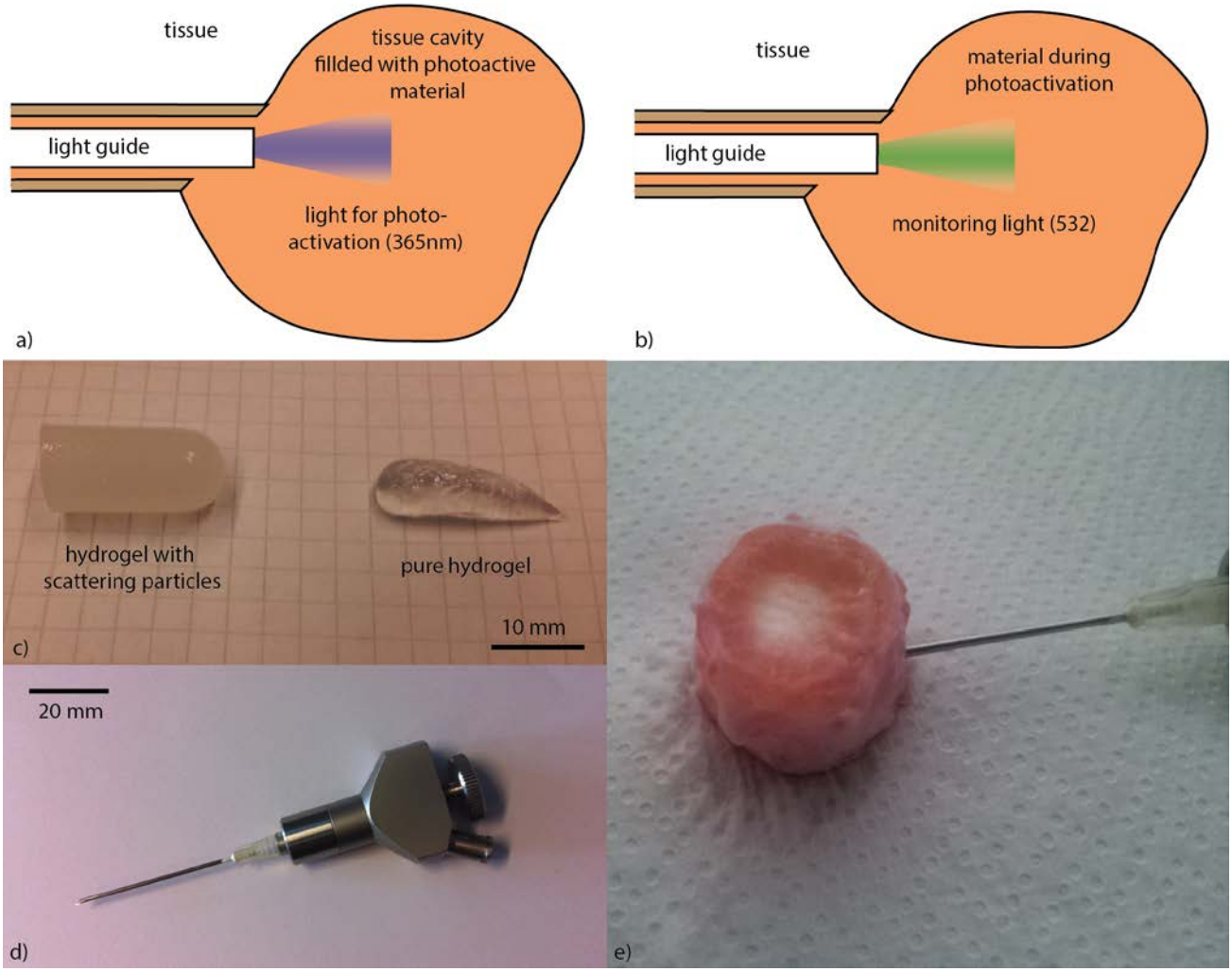

Figure 2a) To induce the photopolymerization reaction ultraviolet light at 365nm is used. b) To track and monitor the reaction green probing light at 532 nm is employed. c) Optically tailored hydrogels with and without scattering lipid particles. Both samples were illuminated with the same fiber. The lipid particles act as a light diffuser within the implant. d) Injection device combining material delivery and optical illumination (further detail in a full SPIE paper[12]). c) Bovine intervertebral disc during surgery and illumination: after injection of a liquid hydrogel the implant is solidified optically using photopolymerization.

The developed procedure did not only able to build up an implant optically within the tissue while providing feedback during the photopolymerization reaction, but could also precisely track the different steps of the surgery. In fig. 3a) a typical Raman and fluorescence spectrum is presented. The Raman peaks, originating from the delivery fiber and is used as a reference. The fluorescence (around 4000 cm^{-1}) originates from tissue scattering implying that the probe is placed closely to tissue. After injection of the inactivated liquid hydrogel the tissue cavity is filled and therefore expands (fig. 3b). This leads to a decreased fluorescence signal which the device was able to track accurately. It could be argued that the signal is due to injected hydrogel. However the hydrogel consists of 90% water and was shown not to contribute to a fluorescence signal in an unpolymerized state. Thus, this decrease of fluorescence signal directly indicates to a surgeon the absence of tissue in the close vicinity of the probe tip – something which has been shown to be essential for a successful photopolymerization[12].

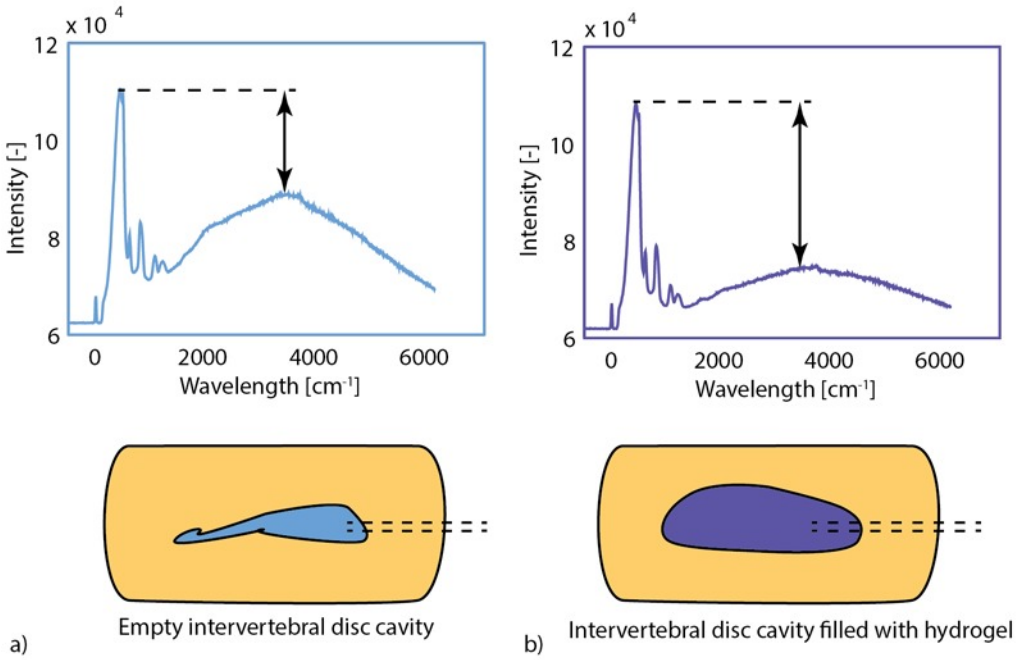

Figure 3 Raman and fluorescence spectra are influence by the position of the probe (dashed parallel lines) and the shape of a tissue cavity: a) the probe is placed within an empty cavity. The fluorescence signal indicates to the surgeon the proximity of the surrounding tissue. b) After injection of a photoactive hydrogel the signal decreases indicating the absence of tissue fragments close to the probe tip. This is important because the fragment would inhibit the photopolymerization reaction.

4. BIOCOMPATIBILITY TESTING

To demonstrate the translational potential of the technique, it is necessary to prove that it does not damage or affect the organ (in this case the intervertebral disc) and the surrounding cells. Thus, biocompatibility tests were conducted. In fig. 4 the results of a Celltiter assay (Promega, Dübendorf, Switzerland) illustration the reaction of nucleus pulposus cells. These nucleus pulposus cells are present within the intervertebral disc and would be directly affected by an implant or the surgical procedure. The biocompatibility of liquid and solid hydrogels was evaluated. This is required because the liquid hydrogel can diffuse into the tissue and might not photopolymerize completely. The solid hydrogel provides information on the long-term biocompatibility of the implant.

The liquid hydrogel mixed with culture medium (“50%”) did not show a significant impact on the cell viability at 24, 48 and 72 hours compared to the “control”. The amount of living cells within the solid hydrogel (“gel”) dropped compared to the “control” at the beginning (24h) – although still being significantly higher than the “negative control”. In any case, over time it was able to recover and at 72 hours of incubation there was no significant difference between “gel” and “control” anymore. This indicates that the procedure has a limited impact on the cell viability of intervertebral disc cells.

However, the material is not toxic and on the long run the cells are able to survive next to the implant without being damaged.

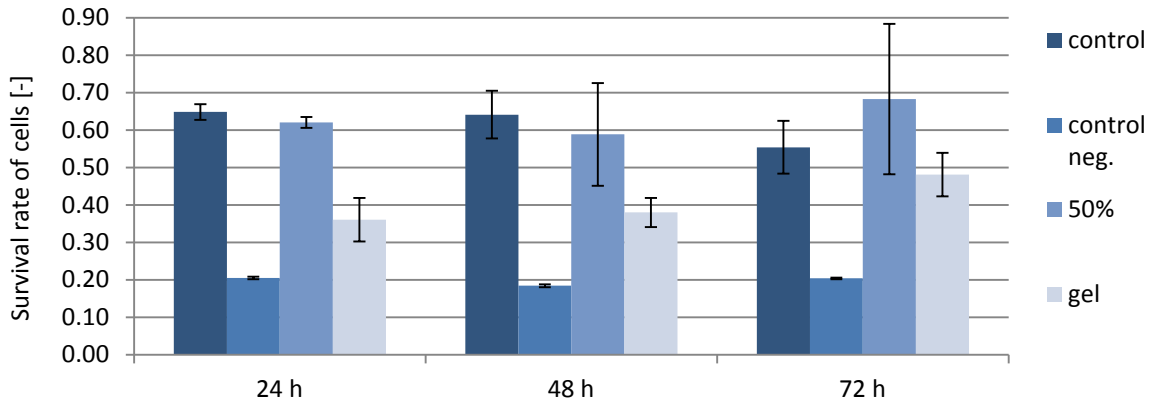

Figure 4 Celltiter assay evaluation the biocompatibility of the implant. A "control" (cells only – no hydrogel added) and a "negative control" (no cells) were used as benchmarks. For the first test ("50%") unpolymerized liquid hydrogel was directly mixed with cells suspended in medium at a volume ratio 1:1. For the second test ("gel") cells were directly mixed to the hydrogel and photopolymerized together. The solid samples were then immersed into cell culture medium. All samples were evaluated at 24, 48 and 72 hours. Each test was repeated four times.

5. MECHANICAL LOADING OF AN INTERVERTEBRAL DISD FOR VALIDATION

To validate whether the proposed approach can be translated into clinics, it is necessary to load intervertebral disc mechanically after implantation of the material. Realistic loads within the intervertebral disc are around 2.5 MPa[13]. For this study bovine intervertebral discs were used. If bovine discs are loaded cyclically above ~ 0.8 MPa, loading results in cell death[14]. However, to evaluate an implanted material it is necessary to go up to realistic human loading-parameters and therefore loads around 3 MPa were chosen. Hydrogels were implanted into the intervertebral disc using a 19 Gauge needle and then loaded cyclically. After each 300 cycles the average load was increased by 0.3 MPa (fig. 5a).

After 3000 cycles (fig. 5b) disc heights were reduced significantly by approximately 40 %, but no significant difference was found between healthy intervertebral disc (with real tissue inside) and replaced intervertebral disc (degenerated and replaced with hydrogels). Usually, parts of the tissue should extrude at such loads although the amount of cycles until extrusion might be higher[15]. No extrusion patterns were observed. The intervertebral disc tissue partially extruded, but not for all samples. Also, the gels samples did not extrude, although water or liquid hydrogel was observed at the hole crated by the 19 Gauge needle for most specimens. This illustrates the exceptional strength the *in-situ* photopolymerization procedure was able to provide.

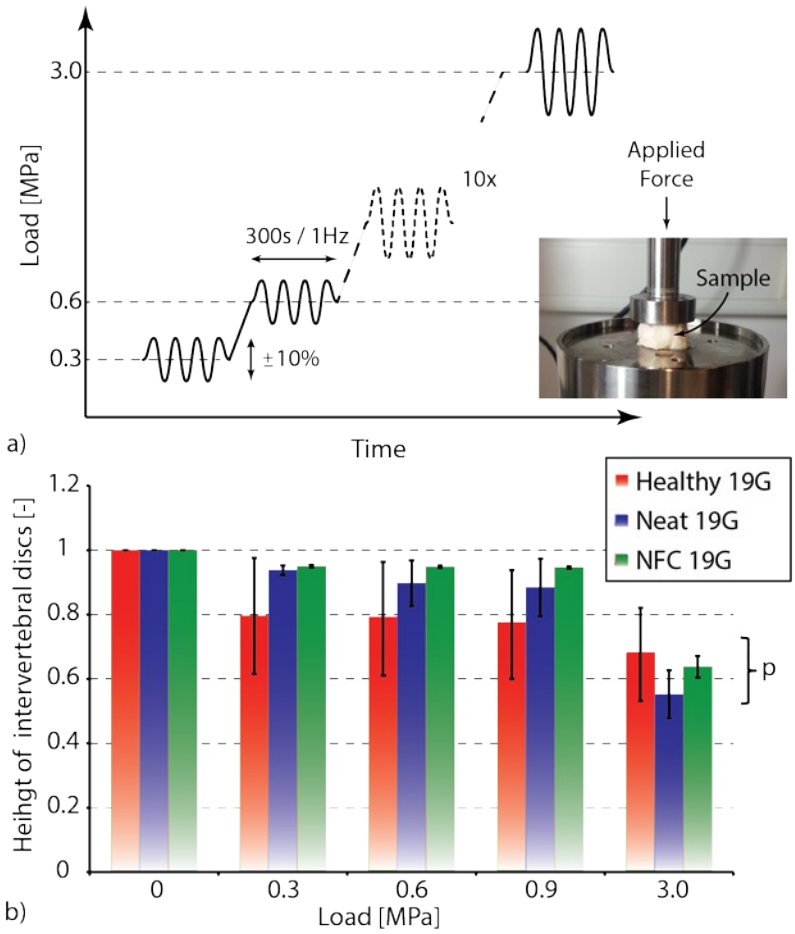

Figure 5 High compressive loading of bovine intervertebral discs up to 3 MPa. a) Protocol: First, a cyclic load of 0.3 ± 0.015 MPa was applied over 300 cycles. Then the load was increased stepwise by 0.3 MPa with a loading amplitude of 10 % of the nominative load (each time 300 cycles) until reaching a load of 3 MPa ± 0.15 MPa. b) Two hydrogels were evaluated in this case: neat hydrogel and hydrogel with an addition of cellulose fibers (NFC). The disc height was measured after each 300 loading cycles. The disc height decreased faster for healthy intervertebral discs, than for intervertebral discs with hydrogel. However, the final disc height was not significantly different p = 0.68 (healthy vs. no NFC) p = 0.26 (healthy vs. NFC). All tests were repeated three times.

6. DISCUSSION AND CONCLUSION

In-situ photopolymerization of implants seems to be an interesting and reliable approach to place materials with the human or animal body. The light activation of the implant allows for complete control. The optical feedback based on Raman and fluorescence spectroscopy provides valuable information on the surgical procedure and the photopolymerization itself. The impact of the surgery onto the surrounding tissue seems to be minimal. After implantation into a bovine intervertebral disc the material is able to reproduce certain functionalities such as the intervertebral disc height over time in a similar manner than a healthy intervertebral disc.

However, certain issues still remain partly unclear, for instance it is possible that if the amount of mechanical loading cycles is increased, at one point the hydrogel might be extruded form the intervertebral discs. Therefore, more extensive testing would be required to properly understand such extrusion mechanisms.

The procedure bears considerable potential for future clinical applications. It is the first procedure to place a liquid implant in a controlled, but also highly minimally invasive manner. For translation into clinics long-term *in-vivo* trials within large animals will be required.

7. AKNOWLEDGEMENT

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