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In vivo testing of a bioresorbable phosphate-based optical fiber / Podrazký, Ondřej; Peterka, Pavel; Kašík, Ivan; Vytykáčová, Soňa; Proboštová, Jana; Mrázek, Jan; Kuneš, Martin; Závalová, Veronika; Radochová, Věra; Lyutakov, Oleksiy; Ceci-Ginistrelli, Edoardo; Pugliese, Diego; Boetti, Nadia G.; Janner, Davide; Milanese, Daniel. - In: JOURNAL OF BIOPHOTONICS. - ISSN 1864-063X. - 12:7(2019), p. e201800397. [10.1002/jbio.201800397]

Availability:

This version is available at: 11696/77354 since: 2023-09-21T12:16:50Z

Publisher:

Wiley

Published

DOI:10.1002/jbio.201800397

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***In-vivo* testing of a bioresorbable phosphate-based optical fiber**

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Keywords: bioresorbable phosphate optical fiber, *in-vivo* testing, pH sensing

Optical fibers have recently attracted a noticeable interest for biomedical applications since they provide a minimally invasive method for *in-vivo* sensing, imaging techniques, deep-tissue photodynamic therapy or optogenetics. The silica optical fibers are the most commonly used because they offer excellent optical properties and they are readily available at a reasonable price. The fused silica is a biocompatible material, but it is not bioresorbable so it does not decompose in the body and the fibers must be ex-planted after *in-vivo* use and their fragments can present a considerable risk to the patient when the fiber breaks. In contrast, optical fibers made of phosphate glasses can bring many benefits, because such glasses exhibit good transparency in Ultraviolet-Visible (UV-Vis) and near-infrared (NIR) regions and their solubility in water can be tailored by changing the chemical composition.

The bioresorbability and toxicity of phosphate-glass based optical fibers were tested *in-vivo* on male laboratory rats for the first time. The fiber was spliced together with a standard graded-index multi-mode fiber pigtail and an optical probe for *in-vitro* pH measurement was prepared by the immobilization of a fluorescent dye on the fiber tip by a sol-gel method in order to demonstrate applicability and compatibility of the fiber with common fiber optics.



Administration of phosphate fibers to a male rat.

1. Introduction

Optical fibers and fiber-based coherent light sources have become increasingly attractive for a wide range of biomedical applications, such as surgical operations, monitoring and chemical analysis of tissues with minimal disruption of the skin or internal organs of the patient [1-3]. However, several key issues in the *in-vivo* application of optical fibers remain up to now unsolved. Among them, one of the most important concerns the material used to fabricate the fiber probes. Indeed, traditionally, fused silica has been the material of choice due to its biocompatibility, high reliability, robustness and outstanding thermo-mechanical properties. These properties make it a great material for fiber-optic probes but it is not bioresorbable, i.e., it cannot be dissolved nor decomposed in the body and therefore the probe construction has to provide protection for the silica fiber in order to minimize the risk of its breakage. The use of such probes for long-term sensing is problematic as well since the risk of the breakage increases with time in the body and the ex-plantation after their use can induce further damage. This issue is critical especially in case of single-fiber imaging of brain tissue [4,5], which is very sensitive.

To tackle the aforementioned criticalities, a bioresorbable optical fiber can be a promising solution [6,7], in particular if such optical fiber is able to degrade under physiological conditions in a limited and controlled amount of time. For the fiber material to be bioresorbable, it must be biocompatible, has to avoid triggering an inflammatory response and has to be eliminated from the body over time without leaving any harmful residual.

Among resorbable materials, inorganic phosphate glasses were demonstrated to be tailorable to provide specific release kinetics [8], optically transparent from the UV to the NIR regions and finally suitable for fiber drawing [7]. Fibers realized with this phosphate bioresorbable glass showed very interesting features and could be employed in applications such as e.g. *in-vivo* sensing using resorbable/disposable devices that do not need explant after use.

While the properties of the bioresorbable phosphate fibers have been extensively tested in a lab environment and a FBG sensor has been realized with them [6], in this paper the *in-vivo* testing of biodegradability and toxicity of the phosphate glass optical fiber is reported for the first time. Moreover, the applicability and compatibility of such fiber is demonstrated with a fiber-optic pH-meter of own design which is based on standard multi-mode silica fibers and was successfully used in the past for *ex-vivo* pH measurement of aqueous humour during cataract surgery [9] or for *in-vivo* measurement of local pH in plant tissues [10].

2. Material and methods

2.1 Fabrication of a bioresorbable phosphate glass fiber

The phosphate-based optical fiber was fabricated by drawing a preform manufactured by the rod-in-tube technique. The core and cladding glasses were synthesized by conventional melt-quenching method using high purity biocompatible chemicals ($P_2O_5 - CaO - Na_2O - SiO_2 - MgO$). Different amounts of MgO and CaO were used to modify the refractive index of the glass. Details of the glass preparation and refractive index dependence on the glass composition can be found in [7].

2.2 Characterization and testing of the bioresorbable phosphate glass fiber

The spectral attenuation of the fabricated fiber was measured by the cut-back method using an ANDO AQ-1425 optical spectrum analyzer (380–1600 nm) and the Fourier-transform infrared (FTIR) spectrometer Nicolet 8700 (1000–2500 nm), adapted for optical fiber input. Fiber samples were excited with tungsten halogen lamp. The solubility tests of the fiber were performed in standard phosphate buffer saline solution (PBS) (Sigma-Aldrich P4417). The tests were performed with buffer solution refreshed every three days as in [7] and with buffer solution kept one month without refreshing. The fiber diameter was measured using the optical microscope Olympus BX-51 equipped with a Complementary Metal Oxide Semiconductor (CMOS) digital camera (IDS UI-1465LE).

The *in-vivo* tests were performed on adult male rats (220-240 g; Wistar Han II, Charles River) in the animal facility of the University of Defense, Faculty of Military Health Sciences and in the laboratories of the Biomedical Research Centre, University Hospital in Hradec Králové. The experiment was approved by the local ethical committee.

The animals were kept in standard lighting (12/12) conditions in plastic breeding cages in the animal facility. The administration of the phosphate fibers was performed in order to evaluate their biodegradability and potential toxicology risk (histopathological tissues analysis, biochemical and hematological analysis of blood). The experiment was designed as follows: 24 animals were divided into 3 groups where each group consisted of 7 experimental and 1 control animals. The experimental animals underwent subcutaneous administration of 1-cm long phosphate fibers (a bundle of 30 fibers). The bundle was secured by two knots of surgical thread as can be seen in Figure 1. A photograph taken during the process of administration of the fibers is shown in the abstract.

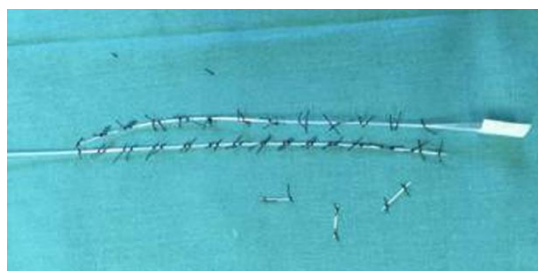


Figure 1 Preparation of the fiber samples for administration.

The control animals underwent “sham” operation, i.e., the same procedure was applied as in the case of the experimental animals but without administration of the fibers. The operation was done in mild isoflurane inhalation anesthesia. The incision (after fiber administration) was provided just by one suture.

Tissue sampling was performed 2, 4 and 5 weeks after fibers administration for the first, second and third group of animals, respectively. The tissue samples were collected after euthanasia of animals conducted by a cervical dislocation in deep fluothane anesthesia. The post-mortem examination included the site of implantation, external surfaces of the body, all orifices of the body and the cranial, thoracic, abdominal and pelvic cavities and their contents. The full set of tissues was collected and fixed as per the Table 1 given below and blood tests for 8 parameters (urea, creatinine, sodium, potassium, chlorides, alanine aminotransferase – ALT, aspartate aminotransferase – AST and C-reactive protein – CRP) were performed. Whole organs or samples of tissue were preserved in 4% neutral buffered formaldehyde. Histological slides were prepared by common paraffin technique and stained with haematoxylin and eosin.

Table 1 Tissue samples collected for histopathology examination.

Tissue/Organ	Fix	Slide	Micropsy
Heart	x	x	x
Lungs (incl. main stem bronchi)	x	x	x
Lymph nodes (axillary, cervical)	x	x	x
Skeletal muscle	x	x	x
Skin with subcutis (site of implantation)	x	x	x
Thymus	x	x	x
Spleen	x	x	x
Liver	x	x	x
Kidneys	x	x	x
Stomach	x	x	x
Duodenum	x	x	x
Jejunum	x	x	x
Colon	x	x	x
All gross lesions	x	x	x

One-way ANOVA, Kruskal-Wallis test with Dunn's Multiple comparison test were selected for statistical evaluation of the blood tests. The skin from the implantation site was histopathologically evaluated on day 14, day 28 and day 35.

Representative samples of the extracted fibers were taken from each group of animals and the minimum and maximum dimensions of the cross-sections were measured in the same way as the samples immersed in PBS solution.

2.3 Demonstration of the bioresorbable phosphate glass fiber in a real fiber-optic sensing system

The developed phosphate glass fiber was tested as a tip of sensing probe for fiber-optic pH meter prototype to demonstrate its compatibility with standard silica optical fibers as well as the applicability in a real fiber-optic sensing system. The prototype exploits the technique of detection of the fluorescence from the pH-sensitive dye - 8-hydroxypyrene-1,3,6-trisulfonic acid, trisodium salt (HPTS) - immobilized at the end of the fiber-optic probe. The probe is based on standard graded-index multi-mode fiber with core/cladding diameters of 50/125 μm which are quite close to the dimensions of the prepared fiber. The principle of operation and preparation of the sensing layers were described in details in [11].

The phosphate glass fiber-optic probe was prepared by splicing a 4 cm-long piece of the bioresorbable fiber with a graded-index silica-based multi-mode fiber with core/cladding diameters of 50/125 μm . The fibers were spliced in a fusion splicer that is designed for splicing standard telecommunication silica-based fibers (Furukawa Fitel S178).

The splice of the silica and phosphate glass fibers was sealed by epoxy resin in a stainless-steel tube for protection purposes (see Figure 2).

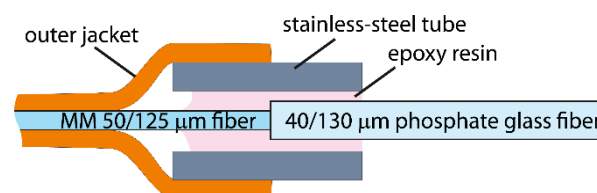


Figure 2 Scheme of the splice of silica and phosphate-based fibers.

Optical probes for pH measurements were prepared by the immobilization of an ion pair of 8-hydroxypyrene-1,3,6-trisulfonic acid, trisodium salt (HPTS) and hexadecyltrimethylammonium bromide (CTAB) on the tip of the fiber by a sol-gel method according to [11]. Since the sensing layer was not originally designed as bioresorbable, the fiber-optic probe was tested only *in-vitro* in a series of Britton-Robinson buffer solutions with pH ranging from 4.3 to 7.5.

3. Results and discussion

3.1 Characteristics of the fabricated phosphate glass fiber

The actual fiber used for pH sensing and in the *in-vivo* experiments showed 40 and 130 μm diameters for the core and cladding, respectively. The measured optical fiber attenuation is reported in Figure 3. The attenuation was found lower than 30 dB/m in the range from 400 to 1600 nm (with a minimum of 7 dB/m at 1300 nm), which is acceptable for the intended application where a short length of fiber is needed.

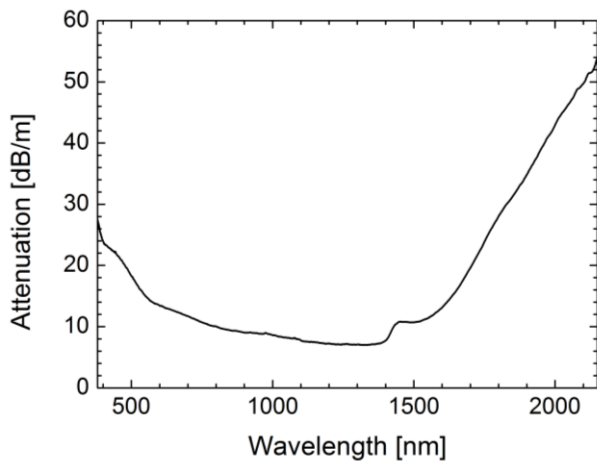


Figure 3 Phosphate glass-based optical fiber attenuation measured by the cut-back method.

3.2 Bioresorbability of the fabricated phosphate glass fiber

The administration sites on the test animals were well healed and the administration of the fibers did not affect the behavior of the animals. They exhibited normal explorative behavior, with no signs of injury. The results of the blood tests are summarized in the Table 2. No clinical signs of adverse effect were found in tested animals upon administration of the phosphate fibers. Namely, the C-reactive protein (CRP), whose levels rise in response to an inflammation, exhibited normal values.

Table 2 Comparison of blood parameters between the different groups of animals.

	Urea	Creatinine	Na	K
CTRL	8.1 \pm 0.0	15.0 \pm 0.0	140.0 \pm 0.0	6.1 \pm 0.0
Group 1	7.0 \pm 1.2	18.0 \pm 1.5	143.0 \pm 1.9	6.0 \pm 0.9
CTRL	7.0 \pm 0.0	20.0 \pm 0.0	144.0 \pm 0.0	6.2 \pm 0.0
Group 2	8.3 \pm 1.0	21.0 \pm 3.1	145.1 \pm 0.7	5.7 \pm 0.7
CTRL	7.1 \pm 0.0	26.0 \pm 0.0	143.0 \pm 0.0	5.7 \pm 0.0
Group 3	7.4 \pm 0.8	22.6 \pm 3.1	143.4 \pm 0.8	6.2 \pm 1.5
Gr.1 vs Gr.2	ns	ns	ns	ns
Gr.1 vs Gr.3	ns	*	ns	ns
Gr.2 vs Gr.3	ns	ns	*	ns
	Cl	ALT	AST	CRP
CTRL	101.0 \pm 0.0	2.5 \pm 0.0	1.6 \pm 0.0	0.3 \pm 0.0
Group 1	98.4 \pm 1.9	1.0 \pm 0.1	1.6 \pm 0.3	0.1 \pm 0.1
CTRL	99.0 \pm 0.0	1.1 \pm 0.0	1.5 \pm 0.0	0.1 \pm 0.0
Group 2	97.7 \pm 1.8	0.9 \pm 0.1	1.4 \pm 0.2	0.2 \pm 0.1
CTRL	102.0 \pm 0.0	1.0 \pm 0.0	1.3 \pm 0.0	0.1 \pm 0.0
Group 3	98.3 \pm 2.7	1.1 \pm 0.2	1.6 \pm 0.2	0.2 \pm 0.1
Gr.1 vs Gr.2	ns	ns	ns	ns
Gr.1 vs Gr.3	ns	ns	ns	ns
Gr.2 vs Gr.3	ns	ns	ns	ns

Group 1 (blood analysis 2 weeks after fibers administration)

Group 2 (blood analysis 4 weeks after fibers administration)

Group 3 (blood analysis 5 weeks after fibers administration)

CTRL (control animals – “sham” operation without fibers administration)

* statistical significance, $p < 0.05$

ns – non-significant

The one way ANOVA, Kruskal-Wallis test with Dunn's multiple comparison test is one of the most frequently used statistical tests for the evaluation of biological data parameters (which are often subject to inter-individual variability) and takes into account, in addition to the obtained values themselves, the scattering or deviation of data within a group. For this reason, the sensitivity of this test then defines the weight of the assessed differences. Only the averages of measured values with variations are shown in Table 2. Mild to minimal fibrosis accompanied by some bands of newly formed capillaries were found in the subcutis of both administered and control animals in all the considered time intervals. The intensity of these lesions was milder in the interval of 28 and 35 days. Further, occurrence of mild focal chronic inflammation in the subcutis was revealed in some administered and one control rat. Finally, there were no considerable differences between administered and control rats.

The other findings revealed in the skin of administered and control animals (scar in dermis, foreign body granuloma, chronic inflammation in dermal muscle, lipogranuloma, epidermoid cyst) were related to the surgical intervention performed. No signs of hepatotoxicity or nephrotoxicity were found in the liver and kidneys parenchyma of the experimental animals after implantation of the test item.

The implanted fiber bundle extracted after 4 weeks is shown in Figure 4 and its microphotograph in Figure 5.



Figure 4 Fibers in the subcutaneous tissue.

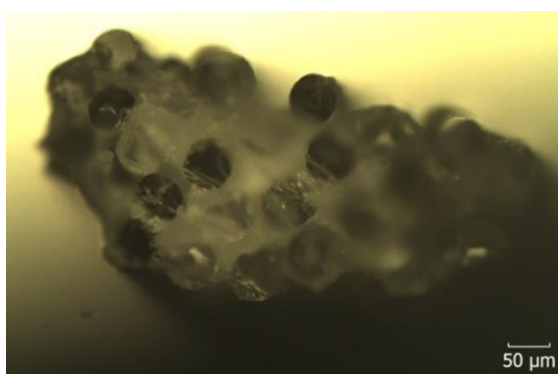


Figure 5 Microscope photograph of the fiber bundle extracted after 4 weeks.

The biodegradability of the fibers in terms of the decrease of their cross-sectional dimensions is depicted in Figure 6.

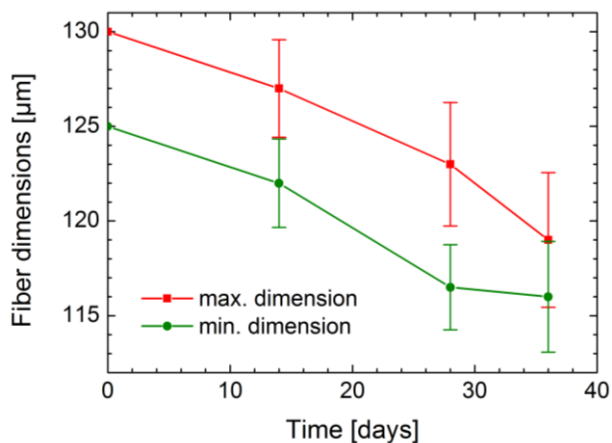


Figure 6 Decrease of the dimensions of the phosphate fibers with increasing length of emplacement under the rat's skin. Since the fiber was slightly elliptical, both maximal and minimal cross-sectional dimensions of the extracted fibers were measured.

The rate of bioresorbability *in-vivo* was found to be slower (0.3 $\mu\text{m}/\text{day}$) than that previously measured *in-vitro* (1.4 $\mu\text{m}/\text{day}$) using regularly refreshed PBS solution [7]. This was a rather unexpected finding, therefore it was decided

to replicate the dissolution experiment *in-vitro* by using a PBS solution both refreshed every three days as in [7] and kept one month without refreshing. Dissolution rate values of 1.4 and 0.4 $\mu\text{m}/\text{day}$ were assessed for refreshed solution and the same solution kept for a month, respectively, thus proving that the solubility of the fibers significantly depends on the refreshing conditions of the PBS solution. In light of all these considerations, the slower solubility *in-vivo* can be ascribed to the encapsulation of the fiber bundle in the animal body that decreased the contact of the external object with freshened physiological fluid inside the animal. Such reaction of the animal body to the object indicates that the bioresorbability of the material of the optical fibers shall be designed with an even faster bioresorbable rate.

3.3 Demonstration of the bioresorbable phosphate glass fiber in a pH sensing fiber-optic

The splicing experiments showed that the fibers had to be aligned in a position about 300 μm away from the center of the electrical discharge and splicing was performed using lowered discharge current. The fibers before and after splicing are shown in Figures 7a and 7b, respectively.

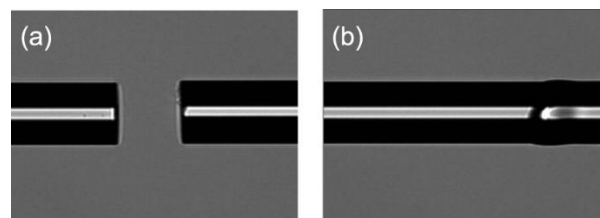


Figure 7 Preparation of the sensing probe based on the phosphate glass fiber: (a) Silica and phosphate fibers before alignment and splicing; the phosphate glass fiber is on the right. (b) Silica and phosphate fibers after splicing.

Since the phosphate-based fiber exhibited a much lower melting temperature than the silica fiber, the phosphate glass material had risen over the silica fiber, as evident in Figure 7b.

The tip of the prepared fiber-optic probe for use with pH-meter prototype with the sensitive layer deposited at the end-face of the fiber is shown in Figure 8.

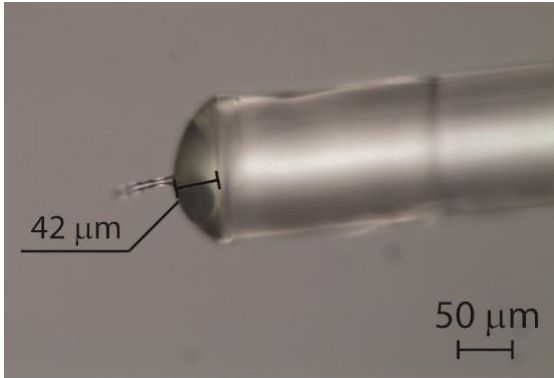


Figure 8 Sensing layer deposited on the perpendicularly cleaved end-face of the phosphate glass fiber.

The time response of the sensor in Britton-Robinson buffer solutions in the pH range from 4.3 to 7.5 is shown in Figure 9a. It was fitted with Boltzmann's equation:

$$y = A_2 + \frac{A_1 - A_2}{1 + e^{\frac{x - x_0}{\Delta x}}} \quad (1)$$

The sensor response curve fitted by Boltzmann's equation is reported in Figure 9b and the parameters of the equation are summarized in Table 3.

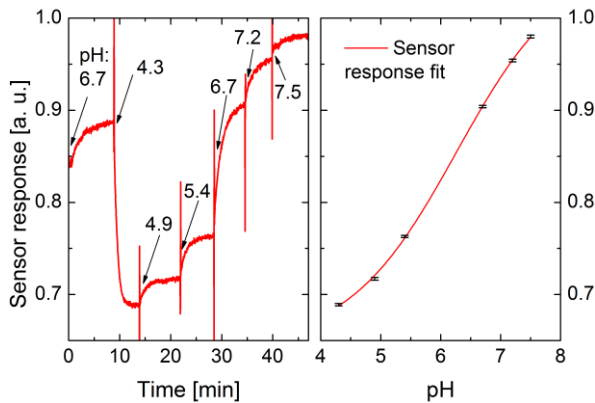


Figure 9 Sensor response to Britton-Robinson buffer solutions in the pH range from 4.3 to 7.5: (a) Response in time. The time instants of the respective solution changes are denoted by arrows. (b) Sensor response fitted by Boltzmann's equation

Table 3 Boltzmann's equation parameters.

Parameter	Value	Standard error
A_1	0.64556	0.01158
A_2	1.06343	0.02409
x_0	6.26266	0.09410
Δx	0.89841	0.09334

The optimal measurement range of the sensor revealed to be from pH 5.0 to 7.0. A slight drift of the signal of 3%/h observed during the pH measurement was caused by drifting of the output power of the 405 nm laser diode in the pH-meter prototype. The response times were of the

order of minutes due to the thicker sensing layer compared to the tapered silica probes used in [9,10].

4. Conclusion

In conclusion, a multi-mode bioresorbable phosphate-based optical fiber with core and cladding diameters of 40 and 130 μm , respectively, was successfully designed and developed. The fiber showed an attenuation loss lower than 30 dB/m in the range 400-1600 nm and proved to be resorbable both *in-vitro* in physiological saline solution refreshed every three days and *in-vivo* in adult male rats, with dissolution rates of 1.4 and 0.3 $\mu\text{m}/\text{day}$, respectively. The slower solubility *in-vivo* was ascribed to the encapsulation of the fiber bundle in the animal body, which is responsible for the decrease of the contact of the external object with freshened physiological fluid. The *in-vivo* test was performed administrating a bundle of 30 fibers with length of 1 cm to adult male rats for a period of maximum 5 weeks. The histopathological tissues and the blood of the animals were thoroughly analyzed at the end of the test and no clinical signs of adverse effects were revealed. Moreover, the bioresorbable phosphate-based optical fiber was employed as a pH sensing probe in physiological environment. Within the probe preparation, splicing of fibers composed of materials with thermal expansion coefficients approximately one order of magnitude different (0.6×10^{-6} and $10 \times 10^{-6} \text{ }^\circ\text{C}^{-1}$ for silica and phosphate glasses, respectively) was demonstrated. Good performance of the probe was demonstrated in Britton-Robinson buffer solutions and an optimal response to pH in the range from 5.0 to 7.0 with response time of the order of minutes was obtained.

Acknowledgements The work was supported by the Agency for Healthcare Research of the Czech Republic under project No. 15-33459A; European Action COST MP1401 "Advanced Fiber Laser and Coherent Source as tools for Society, Manufacturing and Lifescience"; Interdepartmental Centre "PhotoNext" of Politecnico di Torino.

Portions of this work were presented at the International Conference Optical Fibers and Sensors for Medical Diagnostics and Treatment Applications XVIII, part of the symposium SPIE Photonics West BiOS that took place between 27 January and 1 February 2018 in San Francisco, USA [12]. The authors would like to thank Pavel Zitta for the verification of the *in-vitro* degradability tests.

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Graphical Abstract for Table of Contents

Text: Bioresorbable optical fibers can be successfully employed for long-term *in-vivo* sensing, photodynamic therapy or optogenetics because they decompose in the body over time and thus they do not require ex-plantation after usage. Phosphate-glass based bioresorbable optical fibers offer good optical properties and tailorable resorbability rate on the basis of the material composition. *In-vivo* bioresorbability tests of such fibers are presented for the first time and their applicability is demonstrated *in-vitro*.

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