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ARTICLE

Chicken albumen based whispering gallery mode microlasers

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Microlasers based on biomaterial has attracted enormous interest because of their promising potentials for future applications in medical treatments, bio-tracking, and biosensing. In this work, we demonstrate chicken albumen as a novel and excellent low-cost biomaterial for a laser cavity. By using a simple but effective emulsion process, rhodamine B doped chicken albumen microspheres with various diameters ranging from 20 μm to 100 μm can be fabricated. Under optical pulse excitation, these microspheres emit lasing emission. The lasing mechanism is investigated and ascribed to whispering gallery mode (WGM). A threshold of 23.2 μJ/mm^2 and a high Q-factor of approximately 2400 are obtained from a 82 μm-diameter microsphere. Size-dependence lasing characteristics are also examined, and the result shows a good agreement with the WGM theory. Interestingly, these microsphere biolasers can operate in aqueous and biological environments such as water and human blood serum which makes them a promising candidate for laser based biosensing and biological applications.

1. Introduction

Whispering gallery mode (WGM) based microlasers have drawn tremendous attention due to their remarkable properties such as low lasing threshold, high quality (Q) factor, small volume[1] and simple fabrication processes.[2,3] In biological and medical applications, WGM based microlasers have been successfully implanted in multiple biological medium[4–6] for ultra-sensitive sensing[7–10] and cell tracking.[4,11] In these applications, utilizing biomaterials for both gain and laser cavity are always of high interest due to their excellent environmental compatibility and good biocompatibility. Some bio-extracted materials such as bovine serum albumin,[12,13] pectin[13] and silk fibroin[14,15] were successfully implemented as laser cavities. Due to their biological origin, these microlasers, the so-called biolasers, exhibit good biocompatibility while still owning excellent lasing properties.

However, bio-extracted materials are normally costly since they are required complicated extraction processes for a high purification. This limits the future applications of biolasers. To solve this issue, non-extracted biomaterials or natural biomaterials such as human tissues,[16] whole blood[17] and potato starch[18] are recently investigated for biolasers as they do not require chemical synthesis and always available. Particularly, Wei et. al. demonstrated for the first time a microlaser utilizing potato starch as a cavity laser.[18] Even though the microlaser demonstrated good lasing performance, the laser still suffered from a drawback of low Q factor (of ~1000) and high lasing threshold which might come from the medium optical transparency of starch. A poor mechanical property of starch is another disadvantage of the starch based microlasers.[19,20] Therefore, exploring novel and cost-effectiveness natural biomaterials for biolasers is always of high demand.

Among natural proteins, chicken albumen is a promising material for biolasers. Chicken albumen has been widely used in numerous medical and electrical applications. For example, chicken albumen was used in immunological studies as a carrier protein in vaccines and a model protein in chicken sensitivity allergy tests.[21] Chicken albumen was also effectively applied as a gate dielectric in the organic field effect transistor.[22] For optical applications, chicken albumen is a promising material due to its high solubility in aqueous media, good optical transparency. For instance, Wang et al. used chicken egg white as an excited donor to overcome the low UV-light-emitting of SnO_2 nanowire resulting in an enhancement in lasing operation.[23] Comparing with other biomaterials such as bovine serum albumen, pectin, chicken albumen has a lower cost, higher refractive index and it is easily accessible. These advanced properties make chicken egg albumen an excellent material for high Q factor laser cavity. Nevertheless, natural chicken albumen has not been investigated as an appropriate material for a laser cavity.

In this work, we demonstrate natural chicken albumen as a novel and excellent material for biological microsphere lasers. These microlasers are fabricated by a simple but effective method. They have a relatively low threshold, high Q factor and can work well in biological environments such as water and human blood serum.

2. Experimental

Reagents and chemicals

Rhodamine B (RhB), and ethyl acetate ($CH_3COOC_2H_5$) were purchased from Sigma-Aldrich. Polydimethylsiloxane (PDMS) (the base material of Sylgard 184 Silicon Elastomer) was purchased from Dow Corning. A fresh chicken egg was purchased from our local

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market. The chicken albumen solution was extracted directly from the chicken egg without purification. The human blood serum was provided by our local hospital.

The dye-doped chicken albumen solution was obtained by mixing 20 μL RhB 1 wt% solution with 50 mg un-treatment chicken albumen (protein account for 10 wt%).[24] The dry ratio of proteins and RhB in microsphere lasers is estimated as 98 wt% and 2 wt%, respectively. It is noted that organic fluorescent dye is a well-known laser material due to its high quantum well efficiency and broad emission.[25] Herein, organic dye rhodamine B was chosen as the gain material because it is highly absorbed in 532 nm and can be excited by a common Nd: YAG laser.[26]

Dye-doped chicken albumen microsphere preparation

A drop of dye-doped chicken albumen solution was injected slowly into PDMS resin (Figure 1a). Then, a micro needle was implemented to split the droplet into smaller micro-size droplets (Figure 1b). Since the dye-doped solution is immiscible in PDMS, the microsphere droplets were self-assembly formed because of the surface tension. The droplets were then shrunk down and self-solidified by gradually heating the PDMS at 80°C for 30 minutes by a hotplate (Figure 1c). Finally, dye-doped chicken albumen microspheres were obtained after removing the PDMS resin by ethyl acetate solvent (Figure 1d). The obtained microspheres were kept in ethyl acetate (to reduce the oxidation of dye molecules) for further optical investigations.

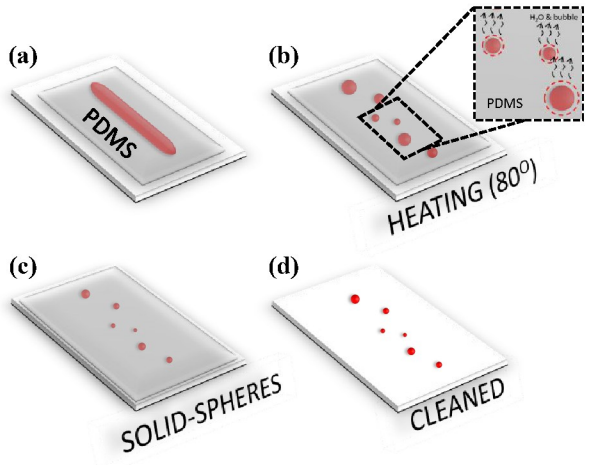

Fig. 1 Schematic diagram of the fabrication process of dye-doped chicken albumen microspheres.

It is noted that the temperature and time were optimized based on the experiment. In principle, if the droplets are heated at a higher temperature (such as 100 °C), it will take a shorter time to solidify thus the fabrication process can be faster. However, high temperature will lead to strong evaporation of water vapour that causes some defects on the microsphere surface. As a result, a temperature lower than 100 °C is preferable. During the experiment, we have found that 80 °C is a suitable temperature and 30 minutes is sufficient time to evaporate all water from the initial droplets and solid microspheres with smooth surfaces can be obtained.

Optical characterizations

The lasing emission from each microsphere was investigated by a micro-photoluminescent setup at room temperature and ambient humidity. The setup includes a 532 nm Nd: YAG nanosecond laser (Litron Laser, repetition rate of 10 Hz, pulse duration of 7 ns) operated as a pumping source, a microscope (Amscope), a spectrometer (AvaSpec-2048L, Avantes), and a camera. The dye-doped chicken albumen microspheres, placed on a highly transparent slide of glass, were excited under different energies. The exited beam spot size was about 350 μm in diameter. The lasing emission was collected by the objective lens (10X of magnification, NA=0.25) of the microscope, guided to the camera for top view image capture, and to the spectrometer for spectral analysis. The spectrometer's resolution is about 0.2 nm. In addition, the surface morphologies of the fabricated microspheres were investigated by the same optical microscope (Amscope) and by a scanning electron microscope (SEM, NOVA NANOSEM 450).

3. Results and discussion

Dye-doped chicken albumen microspheres

Dye-doped chicken albumen microspheres with diameter from 20 μm to around 100 μm exhibit uniform colour, spherical shapes. Figure 2a and 2b show the optical images of the microspheres observing at different angles of 0° and 30° with respect to the parallel plane of the microscope's translation stage. The microspheres demonstrate circular shapes at different angles which confirms the spherical shapes of the microlasers. In addition, the microspheres exhibit relatively smooth surfaces verified by SEM images (Figure 2c, 2d). These two important aspects are necessary for high optical confinement and thus low lasing threshold can be achieved.

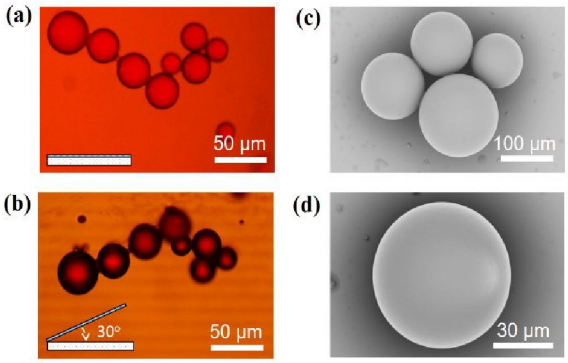

Fig. 2 (a) and (b) Optical images of the dye-doped microspheres at 0° and 30° with respect to the parallel plane of the microscope's translation stage, respectively. (c) and (d) are low and high magnification SEM images of the microspheres, respectively.

Chicken albumen based microlasers in the air

Figure 3a demonstrates the emission spectra from a 82 μm microsphere when the pump pulse fluence (PPF) increases from 13.4 μJ/mm^2 to 73.6 μJ/mm^2. In this excitation range, the transition from spontaneous (broad-spectrum) to lasing emission (narrow peaks) can be observed. When the PPF reaches over the threshold, a number of

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sharp, and high intensity lasing modes are achieved. As shown in the inset, the full width at half maximum (FWHM) of a lasing mode at pump fluence of 38.2 μJ/mm² is 0.26 nm. The Q-factor of the microsphere, defined as Q=$\lambda/\delta\lambda$, is approximately 2400 which is comparable with WGM microdisks reported with different fabrication method.[27] Compared with starch based microlaser, the microsphere laser exhibit a significantly higher Q factor.[18]

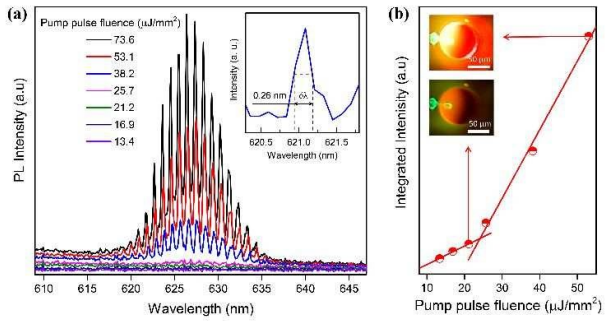

Fig. 3 (a) Emission spectrum observed from a 82 μm dye-doped chicken albumen microsphere illuminated at different PPF values. The inset shows the FWHM of the microlaser's spectrum under 38.2 μJ/mm². (b) The integrated intensity values as a function of PPF. The insets show the optical images of the microspheres at different PPF.

Figure 3b presents the integrated emission intensity as a function of PPF showing a nonlinear dependence which is the evidence of a lasing threshold. The lasing threshold is determined to be around 23 μJ/mm². This value is comparable with other microsphere lasers[12,13,28] and lower than starch based microlasers.[18]

The lasing wavelength can be well fitted with WGM theory. Lasing emission from a WGM microsphere laser can be either transverse electric (TE) or transverse magnetic (TM). It has been demonstrated that Q factor of TE modes of a microsphere laser is expected to be higher than TM modes[29] thus the lasing peaks are supposed to be TE modes. Assuming the radial mode number $r = 1$ as it has the lowest lasing threshold.[13] The mode number at the resonant wavelength λ_m can be calculated as $m = \pi Dn/\lambda_m$ where m is the mode number, D and n are diameter and refractive index of the microsphere, respectively. With D = 82 μm, n = 1.51 estimated from the lasing spectrum (will be discussed later), the mode number corresponding for the lasing wavelengths can be calculated to be 613 to 626 (Figure 4). The result shows a good agreement between experimental observation and theoretical prediction.

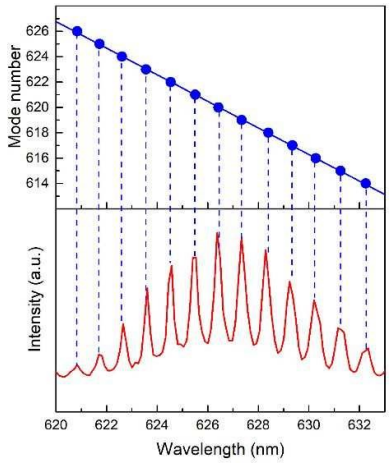

Fig. 4 Matching experimental observation lasing wavelengths and calculated lasing modes of a 82 μm dye-doped chicken albumen microsphere.

In addition, the lasing of the microspheres under continuous pumping is also studied. It has been shown that a 65 μm-diameter microsphere still can lase under excitation pulses of 2.7×10³. This confirms the stability of our microlaser.

Size-dependence lasing characteristics

Free spectral range (FSR) of a microlaser, defined as the spectral distance between two adjacent modes, can be well modulated by varying its size. Figure 5a shows that when the laser diameter decreases, the number of lasing modes also decreases and the FSR increases. For a large number of microlasers, a linear dependence between the FSR and the inverse values of their diameters is obtained (Figure 5b). This linear relationship is in good agreement with the theoretical prediction of WGM lasing as $FRS = \lambda^2/\pi nD$[30] and therefore further confirms the WGM mechanism for the lasing action. In addition, based on this linear relation, the effective refractive index of the chicken albumen can be determined to be 1.51. This value is slightly lower than the refractive index of ovalbumin (1.54) - the main protein in chicken albumen.[31] It is understandable because in chicken albumen there are other substances such as proteins (ovotransferrin, ovomucoid, ovoglobulins, ovomucin, and lysozymes), minerals and carbohydrates.

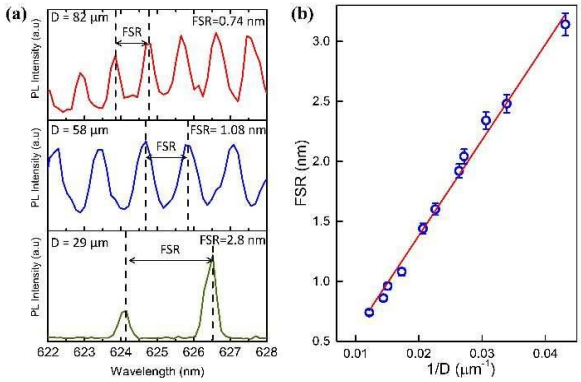

Fig. 5 (a) Spectra of the dye-doped chicken albumen microspheres with different diameters of 29 μm, 58 μm and 82 μm, respectively. (b) Free spectral range as a function of 1/D.

Chicken albumen based microlasers in biological fluids

The stability in morphology and lasing emission in different ambient conditions is also an important property that is needed to investigate. The stability of a 62 μm-diameter microlaser is studied by immersing it in different aqueous media such as water and human blood serum at room temperature. The microsphere remains their spherical shape after 2 hours of immersing in water and human blood serum. No hydration, swelling was observed. More importantly, during this time, the microsphere still lazes in these biological fluids

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with sharp and high intensity modes (Figure 6a). However, it worth noting that the lasing intensity tends to decrease after two hours.

In comparison with the laser in air, the lasing wavelengths are blue-shifted when the laser is immersed in water and human blood serum. Lasing wavelengths of around 62 µm-diameter microlasers in the air appear from 615 nm to 635 nm but they are shifted to shorter wavelengths, from 592 nm to 612 nm when the laser is immersed in water, and from 593 nm to 600 nm when the laser is immersed in human blood serum. The blue-shift of lasing wavelengths is due to the broad emission of RhB and the change in optical confinement. The microsphere laser in the air has the highest optical confinement or highest Q factor thus only lasing emission with long wavelengths, around 625 nm, can be coupled out and being observed. However, when lasers immersed in water or human serum, the refractive index of the outside of the microspheres increase leading to lower optical confinement and lasing wavelengths shift to shorter wavelengths. This effect is quite common and it has been observed previously.[26]

The change in the refractive index of the surrounding medium also affects lasing threshold (Figure 6b). Lasing threshold of the laser is lowest (30 µJ/mm²) when it is in the air and highest (65 µJ/mm²) when it is in the blood serum. It is noted that not only water presenting in human blood serum but there are also many other substances such as cells, proteins fragments, ions, glucose, cholesterol, electrolyte etc. These components create an effective refractive surrounding the microsphere and corresponding for the shift of lasing wavelengths and the increase of lasing threshold.

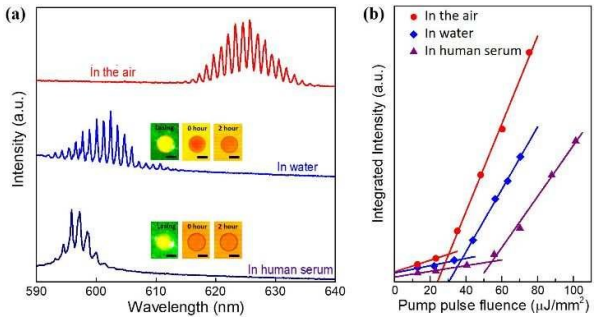

Fig 6 (a) Lasing emissions of dye-doped chicken albumen microspheres in air, in water, and in human blood serum. The insets show the microlaser under the optical excitation and the spherical shapes of the samples in the corresponding aqueous environments. The scale bars are 30 µm. (b) The integrated intensity value as a function of PPF of the dye-doped chicken albumen microsphere in air, in water and in human serum.

The unchanged shape and the stable lasing emission of the fabricated microspheres after a few hours of immersing in different biological fluids such as water and human blood serum confirm the stability of the microlaser. Therefore, we believe that the dye doped chicken albumen based microlasers are applicable for biological and medical sensing. Furthermore, their natural origin also allows them to be implanted in biological environments such as living cells or tissues for tracing of biological activities. This kind of biological integration is an interesting topic and we plan to investigate it in future work.

Conclusions

We have successfully fabricated dye-doped chicken albumen microsphere lasers by using a simple emulsion process. These lasers generate lasing emission with a lasing threshold of a few tens of µJ/mm² and they exhibit high Q-factor of 2400. The lasing mechanism is investigated and is ascribed to whispering gallery mode. Free spectral range and lasing mode position of the microlasers are also investigated and the results show a good agreement with theoretical calculation. Furthermore, the microlasers can lase well when they are immersed in biological fluids such as water and human blood serum for around 2 hours. This result indicates that chicken albumen based microlasers are promising for biosensing applications and bio-integration.

Conflicts of interest

There are no conflicts to declare

Acknowledgements

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Chicken albumen based biological microlasers are fabricated and these biolasers can work in the air, water and human serum.