

PACS numbers: 42.62.Be; 87.85.J–; 87.85.Qr; 87.85.Rs
DOI: 10.1070/QE2014v044n07ABEH015484

Laser-activated nano-biomaterials for tissue repair and controlled drug release

P. Matteini, F. Ratto, F. Rossi, R. Pini

Abstract. **We present recent achievements of minimally invasive welding of biological tissue and controlled drug release based on laser-activated nano-biomaterials. In particular, we consider new advancements in the biomedical application of near-IR absorbing gold nano-chromophores as an original solution for the photothermal repair of surgical incisions and as nanotriggers of controlled drug release from hybrid biopolymer scaffolds.**

Keywords: *laser welding, laser–tissue interaction, photothermal properties, chromophores, gold nanoparticles, nanocomposites.*

1. Introduction

Lasers have become a widespread tool in many therapeutic applications and treatments in the medical field. Minimal invasiveness as a main distinctive feature of laser techniques has provided remarkable advantages, including the possibility to perform therapeutic treatments in the human body at superior levels of localisation and control and to minimise surgical traumas and postoperative complications, which result from the application of sutures and staples. These advantages contribute to improving medical procedures, decreasing healing times, and ultimately promoting the quality of life of patients [1, 2].

Lasers emitting near-IR (NIR) light are used in medicine to induce surgical or therapeutic effects due to the good transmittance of biological tissues in a window between 700 and 1300 nm [3]. Thanks to the poor absorbance of the main components of biological tissues such as melanin, haemoglobin and collagen, NIR light penetrates deep into the body and minimises collateral damage [1]. Remarkable examples of the biomedical use of lasers emitting NIR light include, among others, sutureless bonding and repair of biological tissue and the 'on demand' and localised release of pharmaceuticals. In these cases, the laser treatment is supported by the synergistic use of organic or nano-chromophores [4]. These chromophores generate controlled photothermal effects upon activation with NIR light, which can be modulated with a number of laser parameters such as intensity, irradiation time and modality of light administration. In this paper we present the already consolidated and emerging laser procedures that we have developed at the Institute of Applied Physics (CNR, Italy) in the contexts of the laser repair of biological tissues and laser-activation of drug release from implantable devices.

P. Matteini, F. Ratto, F. Rossi, R. Pini Institute of Applied Physics 'Nello Carrara', National Research Council, via Madonna del Piano 10, 50019 Sesto Fiorentino, Italy; e-mail: R.Pini@ifac.cnr.it

Received 2 March 2014; revision received 4 May 2014
Kvantovaya Elektronika **44** (7) 675–682 (2014)
Submitted in English

2. Laser bonding and repair of biotissues by the use of conventional organic chromophores

The use of lasers to seal accidental wounds and surgical incisions is a valuable and sustainable technology with the potential to replace traditional procedures [3, 5, 6]. Lasers hold the promise to provide instantaneous, watertight seals, which is important in many critical applications such as microsurgery, laparoscopy, endoscopy or for the treatment of extremely thin tissues, for example in gastrointestinal and vascular repairs, without the involvement of foreign suturing materials. Advantages over conventional suturing include shorter operation times, fewer skill requirements, decreased foreign-body reactions and therefore reduced inflammatory responses, increased ability to achieve the regeneration of the original tissue architecture and an improved cosmetic appearance [7].

In this context, the combination of highly penetrating NIR light with corresponding exogenous organic chromophores or dyes, which selectively and locally convert this light into heat, has been become very promising during the last decade [3, 8]. A dye solution is applied where the thermal effects are needed, which is around the edges of a wound or laceration. Currently, two different laser bonding approaches are being pursued, which do or do not involve the addition of a laser-activated glue [7]. In the first approach, usually named laser soldering, an exogenous material such as a protein solution or a polymer preparation is used to convey or improve the adhesive strength. Mixtures of this component and the dye are then placed between the margins of the wound, and finally irradiated with a NIR laser, which drives the thermal activation of the solder [7]. In the second approach, named laser welding, the dye is used to directly stain the open margins of the wound. Then the heat produced upon irradiation with a NIR laser triggers a local thermal modification of the main tissue components such as glycosaminoglycans and collagen (Fig. 1) [9, 10]. In turn, these modifications provide a bond between the adjoining edges of the wound, which results into its prompt repair with minimal formation of scars or risk of tissue rejection. After the treatment, the foreign material including chromophores and glues are resorbed and the wound gradually recovers to its native appearance.

2.1. Laser closure of corneal wounds

Let us consider a few exemplary laser bonding applications that have reached the preclinical and clinical phases and have been developed by our group during the last decade. All these

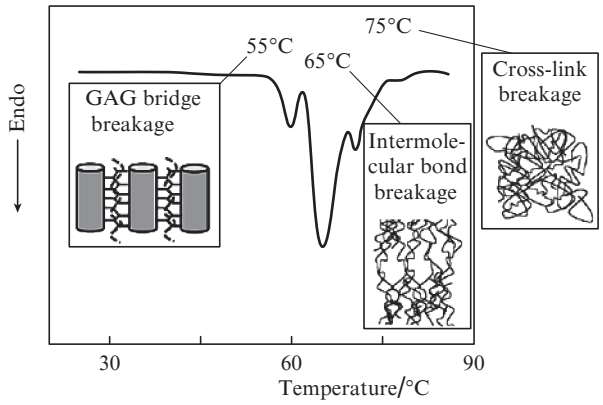

Figure 1. DSC thermogram of a porcine corneal stroma showing three distinct transitions in the 50–80 °C range. Native collagen fibrils are regularly organised in a parallel arrangement with a similar interfibrillar spacing provided by glycosaminoglycan (GAG) bridges. Each fibril features a regular packing of collagen molecules with intramolecular hydrogen bonds (H-bonds) and intermolecular covalent bonds (cross links). As temperature rises above 50°C, the breaking of interfibrillar bridges that impairs the regular fibrillar arrangement occurs. At higher temperatures, the breakage of the intramolecular H-bonds of fibrillar collagen represents the first step of collagen denaturation, which is followed by the breakage of covalent cross-links between and within collagen molecules leading to a full breakdown of the regular peptide assembly and homogenisation of the connective tissue. Adapted from Refs [11–13].

examples are based on the use of a NIR diode laser emitting at 810 nm and the use of the chromophore indocyanine green (ICG), which shows high optical absorption at the laser wavelength. ICG has been chosen because of its biocompatibility, which has already favoured its exploitation in several biomedical applications [14, 15]. The dye is typically applied in the form of an aqueous concentrated solution (10% w/v) of commercially available ICG for biomedical applications (e.g., IC-GREEN Akorn, Buffalo Grove, IL or ICG-Pulsion Medical Systems AG, Germany). This solution is positioned in the tissue area to be welded, using particular care to avoid the accidental staining of surrounding tissues, and thus their detrimental absorption of light. Then the wound edges are approximated and laser welding is performed under a surgical microscope. The laser used in preclinical tests as well as in all clinical applications is an AlGaAs diode laser (Mod. WELD 800 by El.En. SpA, Italy) equipped with a fibre-optic system. The fibre tip is mounted in a handpiece that enables easy handling under the operating microscope and precise delivery of the light beam.

Noncontact, continuous wave (cw) diode laser irradiation has been proposed to weld corneal wounds and cuts, in substitution or possibly in conjunction with traditional suturing procedures [16, 17]. This technology is being applied to human corneal tissues in penetrating keratoplasty (full thickness transplant of the cornea) and lamellar keratoplasty (partial thickness transplant of the cornea) [18]. In the first case, the donor cornea is applied onto the recipient eye and secured by a small number of interrupted stitches, typically between 8 and 16. Then, the ICG solution is placed inside the corneal cut in an attempt to stain its walls in depth. In the second case, a precisely trephined lamellar donor flap is positioned in place to be sutured with the patient cornea, with or sometimes even without application of conventional suture material, and then the wound walls are stained with ICG, as described before. A few minutes after the ICG application, the solution is washed out with abundant water and the whole length of the cut is subjected to laser treatment in substitution of the conventional continuous suturing.

The clinical laser power density in use is around 8 W cm $^{-2}$, which is sufficient to trigger a good welding effect [9]. During irradiation, the fibre tip is kept at a working distance of about 1.5 mm and at an angle of 45° with respect to the corneal surface. This particular fibre position provides in-depth homogenous irradiation of the wound walls and prevents accidental irradiation of deeper ocular structures. The fibre tip is continuously moved over the tissue to be welded, with an overall laser irradiation time of a few minutes.

To date, perforating keratoplasty has been performed on more than 200 patients with very satisfactory results. The position of the apposed margins has been found to remain well stable over time, thus assuring optimal results in terms of postoperatively induced astigmatism after cataract and keratoplasty surgery. In the case of lamellar keratoplasty, intraoperatory observations and follow-up results up to 12 months indicated the formation of a smooth stromal interface, total absence of edema and inflammation and reduced post-operative astigmatism, as compared with conventional suturing procedures.

2.2. Laser-assisted arterial repair and anastomosis

Similar procedures as those described above have been tested *ex vivo* and *in vivo* in animal models in the context of microvascular surgery. It is worth noting that the standard suturing techniques here may be problematic due to the small vessel diameters, while the presence of foreign material may originate severe inflammatory reactions, ultimately leading to thrombosis and occlusion at the anastomotic site [5, 19]. In an attempt to solve these problems, we have developed a laser-based approach involving a soldering formulation composed of ICG and a biopolymer. The aim is the stabilisation of the optical properties of the chromophore over time and the possibility to devise a flexible, biocompatible and easy-to-handle laser-activated glue that may be used in different shapes for specific surgical needs. Our ultimate formulation consists of ICG-infused chitosan films (~40 μm in thickness), which have been tested as soldering tools for laser-assisted vascular repair (LAVR) and anastomosis (LAVA) applications. Here the irradiation procedure combines pulsed laser emission for the tight stabilisation of a film wrapping the vessel (e.g. an artery) and then cw emission to induce the bonding of the film to the vascular tissue [20].

Films are prepared by dispersing chitosan up to a final concentration of 3.5% (w/v) into a weak acidic solution containing 0.02% ICG, followed by pouring in plastic moulds, drying and final water-insolubilisation with alkaline solution [21, 22]. The thus prepared films are resistant, pliable and stable in physiological environment, show a uniform distribution of the chromophore throughout the biopolymeric scaffold and maintain the same optical features for many days after preparation. In a typical procedure, after proximal and distal temporary clipping of a vessel segment, a ~5-mm longitudinal (LAVR) or a full thickness (LAVA) cut across the tissue is performed. Then an ICG-chitosan film is applied onto the lesion, wrapped all around the vessel and then laser soldered in its final position (Fig. 2). In the case of LAVA, three interrupted stitches are still recommended to approximate the vessel stumps and reinforce the anastomosis. Single laser

pulses are then delivered (with typical values of 0.9 W output peak power, 80 ms duration and 100 J cm^{-2} fluence) by gently pressing the fibre tip onto the film while keeping the irradiated zone well hydrated. Single spots are distributed all over the entire surface of the film in order to ensure its fusion to the vessel. Lastly, cw laser irradiation (200 mW, corresponding to 18 W cm^{-2} power density or 200 J cm^{-2} fluence) is performed with an approach resembling those for corneal laser welding, in order to seal and improve the film adhesion to the vascular surface. After surgery, a good reorganisation of vascular-wall structures has been documented, together with a regain of the physiological endothelial morphology in the absence of thrombogenetic effects, both in short- (2 and 7 days) and long-term (30 and 90 days) follow-up periods. In addition, pre- and post-surgery microdoppler analyses have shown that the application of the film does not affect blood flow, even when the vessel is tightly enwrapped.

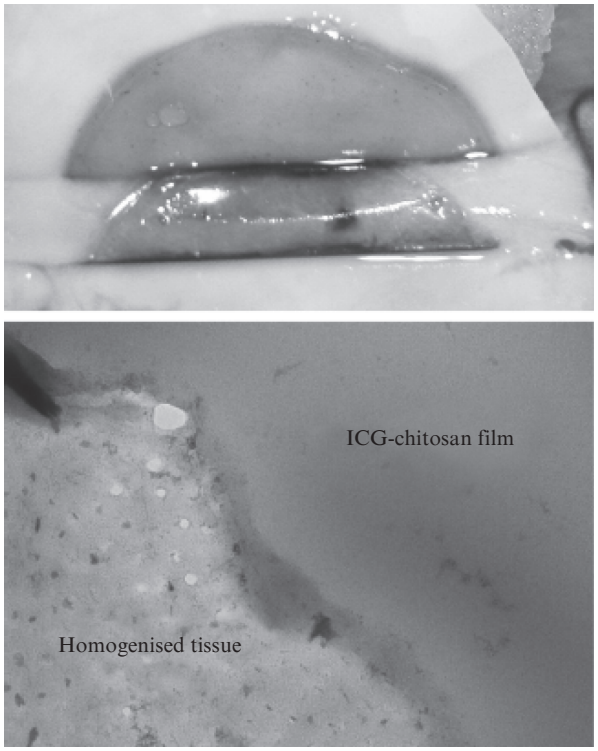

Figure 2. Laser assisted repair of a New Zealand rabbit common artery by pulsed laser illumination of an ICG-chitosan film. Top: wrapping of an ICG-chitosan film over an artery. Bottom: TEM micrograph of the photothermal adhesion line between the ICG-chitosan film and the arterial tissue [20].

3. Laser-bonding with plasmonic nanoparticles

3.1. Plasmonic nano-chromophores

In spite of a large number of surgical applications with a history of safety in humans, organic dyes used in laser treatments may suffer from severe drawbacks such as a limited optical efficiency, low photo-bleaching thresholds, possible phototoxicity (e.g. generation of reactive oxygen species), poor stability in water and problematic diffusivity in the biological environment [14, 23, 24]. In addition, organic dyes exhibit poor chemical versatility, which restricts the choice of formulations in functional compounds, as well as poor optical versatility, which limits their potential for multiplexed applications.

These limitations have motivated recent proposals to replace organic chromophores by plasmonic nanoparticles, which hold the promise to improve and extend the range of applications of laser treatments [23, 25–28]. The broad class of these nanoparticles includes a variety of gold-based constructs, such as the so-called gold nanorods, nanoshells (silica core, gold shell), nanocages and other shapes, with gold nanorods accounting for ~80% of the present literature. The extinction spectra of these nanoparticles display specific fingerprints originating from collective oscillations of free electrons or plasmon resonances and usually comprise a weaker band in the green window, similar to that found for gold nanospheres, and a fairly stronger band at NIR frequencies [29, 30]. The latter can be tuned throughout the NIR window with principal shape parameters, such as the aspect ratio (length divided by waist diameter) of gold nanorods or the ratio between shell and core diameters of gold nanoshells, which can be controlled in their synthesis.

The pursuit of synthetic methods to gain fine control over the size and shape of plasmonic nanoparticles has become an active field of research [31, 32]. Figure 3 shows an example of gold nanorods with similar overall shapes and different average sizes that were prepared at our lab. In turn, after excitation with light from a NIR laser, these plasmon resonances happen to relax mainly through nonradiative channels [33]. The interplay of physical and chemical properties of gold nanoparticles gives a number of favourable conditions such as exceptional optical extinction cross sections (about five orders of magnitude higher than those of organic chromophores [29, 34]), high stability in the body and thresholds

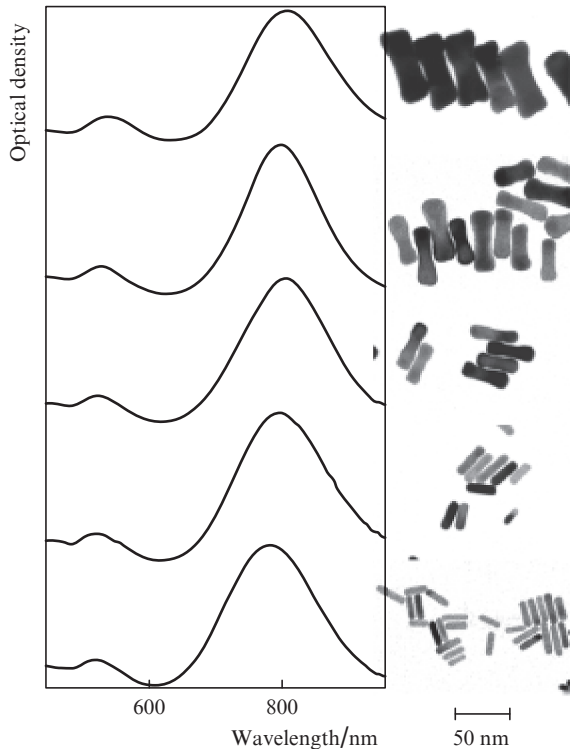

Figure 3. Size control in the synthesis of gold nanorods. (Left): typical optical extinction spectra and (right) representative TEM micrographs of gold nanorods with similar shapes and different sizes.

before photo-bleaching [33], low toxicity [35, 36] and much versatility of conjugation with additional molecules including ligands and drugs. In addition, the potential of particles in a range of size of some tens of nanometres to passively accumulate into tumours after intravenous injection, thanks to their enhanced permeability and retention (EPR), has inspired the use of plasmonic nanoparticles in oncology. Important applications include the photoacoustic imaging [37–39] and optical hyperthermia [40–42] of cancer, which may be performed with the same contrast agents.

In this context, the plasmonic nanoparticles are first modified to reach and stain a tumour often through the blood flow, which requires slow blood clearance and possibly high affinity for neoplastic cells. The former may be pursued by the use of polymeric shells, such as polyethylene glycol that provides a stealth profile against the immune response [43–45]. The latter may be emphasised by the addition of ligands of specific proteins that are overexpressed on the plasmatic membranes of malignant cells, such as the epidermal growth factor receptor [40, 41, 46, 47], vascular endothelial growth factor receptor [40], folate receptor [48–51], etc. Once the plasmonic nanoparticles have stained a tumour, their excitation with short light pulses, often in the nanosecond duration regime from a *Q*-switched laser, produces ultrasounds by photoacoustic effects, i.e. a cascade of photothermal and thermoelastic events, which may be analysed for imaging purposes with the convenience of optical contrast and depth penetration of ultrasonography.

On the other hand, the use of cw lasers may be suitable for a hyperthermia therapy, which is already being evaluated in clinical trials [52]. More recent proposals to remove a tumour by means of plasmonic nanoparticles include the application of short (nanosecond or even femtosecond) light pulses in order to trigger the formation of vapour bubbles and cavitation that may impart lethal damage to individual malignant cells [53], or the addition of cytotoxics, inhibitors, etc. At present the biochemical inertness and poor mobility of gold nanoparticles, which are likely to reside long *in situ* after the treatment with unclear excretion pathways, are perceived as a critical hindrance before their clinical acceptance. However the combination of several features of these nanoparticles, including their high efficiency and stability, biochemical inertness, low toxicity, and also low diffusivity, seem to be ideal for applications in the context of laser bonding, where the highest localisation of the chromophore after topical application is desirable.

3.2. Fabrication and optical tunability of plasmonic nanoparticles

Our group has focused on the design and synthesis of gold nanorods. Aqueous suspensions of these nanoparticles may be synthesised by self-assembly through the reduction of chloroauric acid by ascorbic acid in the presence of the ionic surfactant cetrimonium bromide (CTAB), which forms micelles in the aqueous environment and a tight bilayer around the gold crystals, very small gold nanospheres, which act as nucleation centres, and small aliquots of silver nitrate. During the growth, the adhesion of cetrimonium is more favourable to some facets of the gold crystals than the others, probably due to steric conditions. This preference modulates the supply of gold ions, and so the growth is more rapid along the directions capped by less cetrimonium than the others, which drives the elongation [32, 54]. In turn, the prolate shape is responsible for the appearance of plasmonic bands in the NIR window. After growth, the surface of the gold nanorods may be readily modified by the replacement of cetrimonium with more biocompatible molecules, often through the use of thiols or amines [55].

We have developed a novel procedure to realise a systematic modulation of the size and shape of gold nanorods, based on the control of the fraction and rate of reduction of chloroauric acid by ascorbic acid [32]. In this procedure, we start with the standard method in most common use [56], where the initial growth solution leaves 80% of the gold ions from chloroauric acid only partially reduced and so dissolved or practically unused. Then a gradual addition of ascorbic acid leads to a progressive exhaustion of these gold ions and an overgrowth of the pristine nanoparticles. The rate of addition of ascorbic acid affects fine details of the particle shapes, such as the appearance of either of smooth or so-called dog-bone profiles [33, 57]. In addition to the standard control over particle aspect ratio, which governs the position of the plasmonic bands typically between about 600 and 1200 nm, we note that the particle size plays a key role in parameters such as the ratio of optical scattering and absorbance [29, 32] and the thermal coupling with the environment. The possibility to modulate all these parameters proves useful to optimise many photothermal and photoacoustic applications, including the laser bonding of biological tissue.

3.3. Preliminary tests with plasmonic nanoparticles for laser welding of ophthalmic tissue

As a proof of the concept of the possibility to replace organic dyes with gold nanorods, we tested aqueous batches of these nanoparticles with plasmonic bands in the NIR window overlapping the optical absorption of ICG for the laser welding of a model ocular tissue, that is the porcine eye lens capsule [34, 58]. This is a connective tissue with high transparency and structural homogeneity. Moreover, bonding of capsular flaps remains a critical issue for developing new and remarkable operations in ophthalmic surgery such as the eye lens refilling. In our tests, we simulated the transplant of patches of an eye lens capsule from a donor on the eye lens capsule of a recipient. The interface between these tissues was stained with an aqueous suspension of gold nanorods and then a 810 nm laser light was delivered through a 300-μm-diameter optical fibre. The radiation was given spot-wise in single pulses of 40 ms duration. Under these conditions, we were able to demonstrate reproducible welds with mechanical strength similar to that of native tissues in a range of 80–110 J cm^{-2} fluence. The photothermal effects responsible for sealing together the capsular patches typically displayed a local denaturation of the collagen filaments and consequent macroscopic shrinkage of the tissue by more than 50% of the original thickness. While the use of gold nanoshells for laser bonding of muscles and skin was already reported in combination with an albumin solder [57], for the first time, these tests proved the possibility to trigger functional and well-localised photothermal effects directly in biological tissue by excitation of suitable solutions of gold nanorods.

3.4. Laser-activated nanocomposite gel for the closure of vessels

After a preliminary demonstration of the feasibility of introducing gold nanorods in laser welding practices, our recent

investigation has been focused on a number of drawbacks that can be met in the use of bare colloidal suspensions of these nanoparticles. In particular, several crucial issues in laser welding can be addressed by inclusion of gold nanorods into a biopolymeric matrix [23]. The introduction of a protective barrier against the physiological environment enhances the stability, durability and effectiveness of the chromophore [59]. In addition, nanoparticles are protected against aggregation, which is among the most ubiquitous problems of colloidal formulations in contact with physiological fluids. In turn, aggregates of plasmonic particles suffer from a dramatic loss of their efficiency of photothermal conversion [60, 61]. From the surgical viewpoint, inclusion into a biopolymeric matrix offers additional possibilities of more convenient manipulation and better control over the local density of the chromophore, which is critical for the reproducibility and safety of the laser technique. Moreover, the biopolymer chosen for the fabrication of the nanocomposite can play an active role during the surgery or in the post-surgical period e.g. by inducing a better reorganisation of the tissue, decreasing the formation of scars and even preventing microbial infections. The biopolymeric matrix may also be loaded with drugs or functional molecules, which may optimise or speed up the wound healing process.

In our earlier experience, the surface of gold nanorods was first stabilised with polyethylene glycol (PEG) and then introduced into a hyaluronan gel [62]. The choice of hyaluronan offers the advantage of well-known physiochemical and biological properties and high affinity for biological tissues [63]. The thus obtained nanocomposite gel exhibits viscous and handy appearance and keeps the optical features of the embedded gold nanorods well constant for several months under daylight conditions. We tested this material for the *in vivo* laser assisted vascular closuring (LAVR) of small incisions in the carotid artery of rabbits. For the surgical procedure, the gel was topically applied onto a 3-mm-long longitudinal incision performed on a dissected common artery (Fig. 4). Then the wound was irradiated (810 nm, cw) by means of a 300-μm optical fibre with an optimal power density of 30 W cm^{-2} and an average repairing time of 50 seconds. Doppler flow analysis assured that neither occlusion occurred during the surgery nor after a 30-days follow up. In addition, neither blood leakage nor haemorrhage was observed immediately after the intervention, while collagen and elastic fibres returned to their normal architecture after the follow up [64]. The laser-based approach discussed above is technically easy to perform while the use of gold nanoparticles improves the selectivity of the photothermal effect and minimises the surgical trauma to vessels, resulting in an optimal healing process.

3.5. Laser-activated hybrid bioadhesives for tissue repair

For further optimisation of laser bonding with plasmonic nanoparticles, we tested flexible chitosan films containing a distribution of gold nanorods [65]. Similar to the ICG-chitosan films discussed in Section 2.2, an acidic mixture of gold nanorods and chitosan is poured into moulds and then left to dry in order to obtain resistant, pliable and stable ~40 μm-thick hybrid films. Interestingly the introduction of chitosan and the repeated washing steps used during the fabrication process minimise the concentration of the residual cytotoxic surfactant CTAB to below 50% inhibitory concentration, implying negligible toxicity and potential for safe use in the human body. We point out that the relative concentration of chitosan and gold nanorods needs to be well balanced in order to keep the optical absorbance of the hybrid films close to that of isolated nanoparticles and a suitable efficiency of photothermal conversion. We demonstrated the possibility to use gold nanorods–chitosan films for the laser-assisted repair of arterial tissue. Effective adhesion between the films and explanted patches of porcine carotid artery was obtained with pulsed light (100 ms duration, 100 mJ energy, 140 J cm^{-2} fluence) generated from a NIR laser, which produced spots of local bonds. The adhesion process was studied with a thermocamera together with a computational simulation, which revealed that temperatures around 130 °C are needed at the film–tissue interface in order to activate functional moieties on both sides and achieve satisfactory strength, around 12 kPa [66]. We note that while short femto- to nanosecond pulses are usually responsible for strong heat-confinement within the plasmonic nanoparticles with subsequent reshaping, fragmentation, or even metal sublimation, millisecond pulses such as those used in our protocol minimise unnecessary overheating of individual nanoparticles, thus preventing detrimental side-effects and securing fine control over the photothermal treatment [65].

4. Laser-stimulated drug release from hybrid sponges

An additional possibility of biomedical applications based on laser-activated nanocomposites is a technique that we have recently developed for the controlled delivery of drugs from implantable devices. These devices are typically composed of gold nanorods distributed within a porous chitosan scaffold and also include a dispersion of thermosensitive micelles, which may be preventively loaded with selected drugs (Fig. 5) [67, 68]. The final light-responsive sponge-like composites exploit the concurrence of two principal components, i.e. plasmonic nanoparticles, which readily respond to an exter-

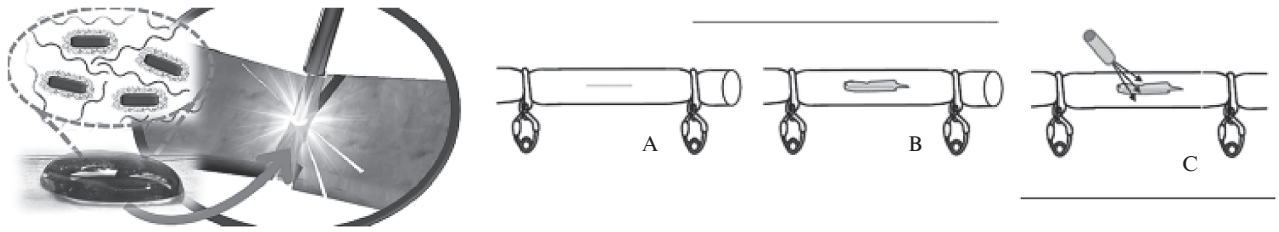

Figure 4. Laser-assisted vascular closuring by laser illumination of a hyaluronan-gold nanorods gel. Gold nanorods are modified with polyethylene glycol and then introduced into a hyaluronan gel, maintaining their pristine photothermal efficiency. After topical application of the gel on the margins of the wound, cw NIR laser illumination delivered by an optical fibre generates effective weld strength.

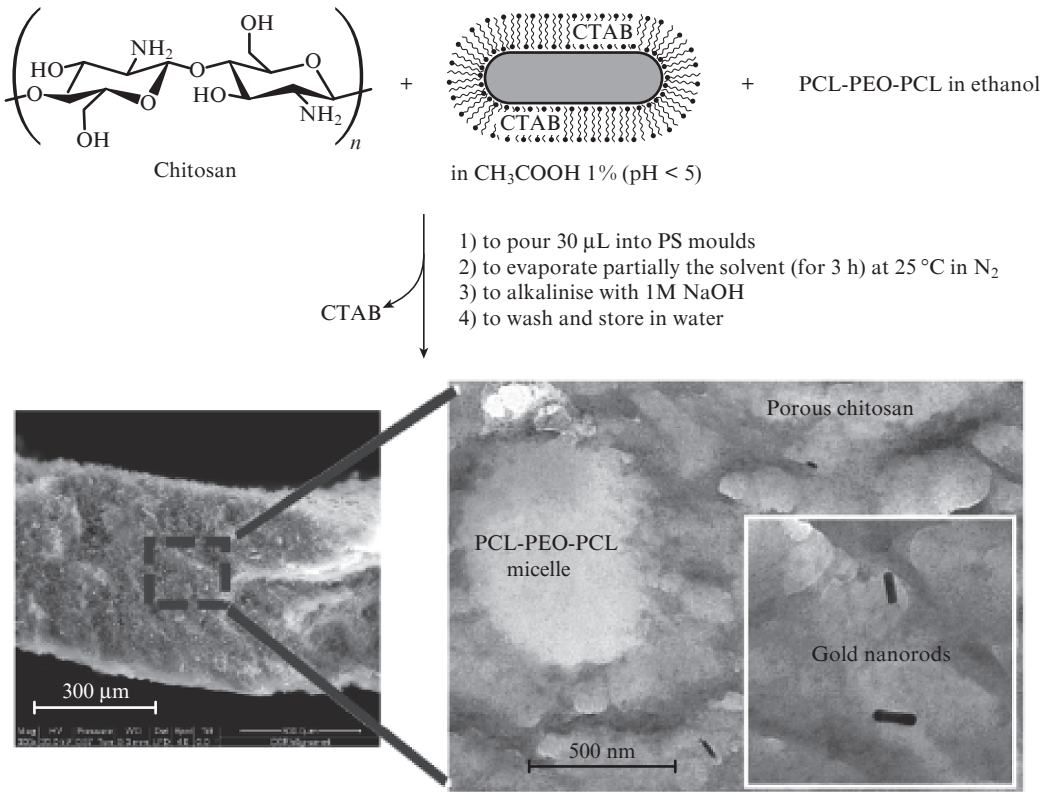

Figure 5. Scheme of fabrication of hybrid sponges (borrowed from Ref. [67]). Chitosan is first dissolved in an aqueous suspension of gold nanorods, followed by the addition of the PCL-PEG-PCL copolymer. The mixture is then poured into moulds and, after 3 h evaporation under controlled conditions, cross-linked with an alkaline solution followed by abundant rinsing with water [67]. Bottom: SEM (on the left) and TEM (on the right) microphotographs of the cross section of sponges (the inset shows an enlarged appearance of gold nanorods dispersed inside a chitosan matrix).

nal optical trigger and produce a well-controlled temperature enhancement, and the micellar drug-carriers, which undergo a modification of their chemo-structural properties upon heating and allow an accurate release of a drug. The latter may be finely modulated with the main irradiation parameters [69]. In particular, we tested micelles of poly(caprolactone-b-ethylene glycol-b-caprolactone, PCL-PEG-PCL), which display excellent payload properties [70]. It is worth noting that these micelles feature a phase transition around 40 °C, which entails a reversible volume contraction by ~35% that is maintained also after their introduction in the porous chitosan scaffold and that triggers the release of the drug from the sponge to the external environment.

We proved the possibility to fine-tune the temperature generated inside the sponge upon cw laser illumination from a 810-nm diode laser with a linear relationship between optical intensity and temperature within a range of interest for the activation of the release mechanism (i.e. slightly above the physiological temperature or ~40 °C to ~50 °C) [1]. Optimal results were obtained by using laser intensities in a range between 0.1 and 0.5 W cm^{-2} and irradiation times of the order of minutes. Immediate outcomes of the temperature control make it possible to prevent superheating effects that may induce irreversible damages in healthy tissues. In turn, accurate temperature tuning provides consistency and control over the dosage of the drug release, which proved to depend on optical intensity and irradiation time. All these features inspire concepts of customised pharmacological treatments that fully exploit the potential of laser-activated nano-biomaterials.

We verified the effective use of our nanocomposites to perform a controlled chemotherapy of cancer cells. Sponges loaded with the chemotherapeutic agent doxorubicin (Dox) were introduced into cultures of HeLa cervical adenocarcinoma cells. By varying the irradiation conditions, we succeeded to induce different yields of cellular death. Larger activation times and optical intensities were shown to provide more effective chemotherapy. As expected, the antitumor Dox activity was enhanced by the synergistic assistance of a hyperthermic effect, which stimulates drug uptake by temporarily increasing the plasmatic membrane fluidity. Moreover, an excellent spatial control over drug release was proven to occur, which we ascribed to a thermally induced hyperpermeability effect experienced by those cells that lay in intimate contact with the laser-heated portion of the sponge.

We finally tested a different configuration in which the sponge was activated by a focused laser beam to release its content to selected areas in a variety of animal tissues, including ophthalmic and gastrointestinal samples. By modulating the irradiation conditions and taking the peculiar optical properties of the different tissues into account, we observed a confined drug release to cellular areas where the sponge was exposed to light. This observation suggests the use of the proposed method to deliver drugs to localised tissue regions with the unique spatial control provided by the laser beam size and a dosage that may be predetermined by modulation of relevant irradiation parameters. For instance these features may be of interest to develop advanced and personalised pharmacological therapies, where a precise release of drugs to specific regions is required.

5. Conclusions

We have discussed some recent advances in the combined use of laser light and organic and nano-chromophores with optical absorbance in the so-called 'therapeutic window' of the NIR spectrum, where light penetration through the body is maximal. In this context, laser-assisted tissue repair is an instructive example. This technology holds the potential to replace traditional surgical procedures thanks to a number of advantages, including the ability of providing instantaneous and watertight seals without the use of foreign suturing materials. Recent progress in the design and synthesis of novel nanoparticles with versatile optical response may improve the current technology of tissue repair based on organic dyes and extend its range of application. Further developments involve the use of hybrid materials by dispersion of the chromophore into a biopolymeric matrix in order to gain ease of use, reproducibility and additional functions. An exemplary case is the introduction of gold nanorods into a chitosan film, which can be precisely secured to a biological tissue by pulsed laser irradiation. When a thermosensitive drug reservoir is added to the nanocomposite, a low-power light stimulation can generate a controlled drug release that can be modulated in space and dosaged by the variation of illumination parameters such as optical intensity and irradiation time. On consideration of the versatility of the pool of applications of plasmonic nanoparticles discussed in this paper, we envisage that forthcoming developments in laser technology and engineering of novel nanocomposites will direct future research toward multifunctional therapeutic platforms, which will introduce concrete benefits into the health care of human beings.

References

1. Niemz M.-H. *Laser-Tissue Interactions: Fundamentals and Applications* (Berlin: Springer, 2004).
2. Menabuoni L., Canovetti A., Rossi F., Malandrini A., Lenzetti I., Pini R. *Acta Ophthalmol.*, **91**, e494 (2013).
3. Pini R., Rossi F., Matteini P., Ratto F. *Laser Tissue Welding in Minimally Invasive Surgery and Microsurgery*, in *Biophotonics* (Berlin: Springer, 2008).
4. Matteini P., Rossi F., Ratto F., Pini R. *Laser Welding of Biological tissue: Mechanisms, Applications and Perspectives*, in *Laser Imaging & Manipulation in Cell Biology* (Berlin: Wiley-VCH, 2010).
5. Esposito G., Rossi F., Matteini P., Puca A., Albanese A., Sabatino G., Maira G., Pini R. *J. Biol. Regul. Homeost. Agents*, **25**, 145 (2011).
6. Bass L.S., Treat M.R. *Lasers Surg. Med.*, **17**, 315 (1995).
7. McNally K.M. *Laser Tissue Welding*, in *Biomedical Photonics Handbook* (Boca Raton: CRC Press, 2003).
8. McNally K.M., Sorg B.S., Welch A.J., Dawes J.M., Owen E.R. *Physics in Medicine and Biology*, **44**, 983 (1999).
9. Matteini P., Rossi F., Menabuoni L., Pini R. *Laser Surg. Med.*, **39**, 597 (2007).
10. Matteini P., Sbrana F., Tiribilli B., Pini R. *Laser Med. Sci.*, **24**, 667 (2009).
11. Matteini P., Cicchi R., Ratto F., Kapsokalyvas D., Rossi F., de Angelis M., Pavone F.S., Pini R. *Biophys. J.*, **103**, 1179 (2012).
12. Rossi F., Matteini P., Bruno I., Nesi P., Pini R. *Opt. Express*, **15**, 11178 (2007).
13. Matteini P., Ratto F., Rossi F., Cicchi R., Stringari C., Kapsokalyvas D., Pavone F.S., Pini R. *Opt. Express*, **17**, 4868 (2009).
14. Landsman M.L., Kwant G., Mook G.A., Zijlstra W.G. *J. Appl. Physiol.*, **40**, 575 (1976).
15. Thaler S., Schuttauf F., Haritoglou C. *Ophthalmologe*, **106**, 11 (2009).
16. Menabuoni L., Pini R., Rossi F., Lenzetti I., Yoo S.H., Parel J.M. *J. Cataract. Refr. Surg.*, **33**, 1608 (2007).
17. Buzzonetti L., Capozzi P., Petrocelli G., Valente P., Petroni S., Menabuoni L., Rossi F., Pini R. *J. Cataract. Refr. Surg.*, **39**, 1829 (2013).
18. Rossi F., Matteini P., Ratto F., Menabuoni L., Lenzetti I., Pini R. *J. Biophotonics*, **1**, 331 (2008).
19. Esposito G., Rossi F., Puca A., Albanese A., Sabatino G., Matteini P., Lofrese G., Maira G., Pini R. *J. Biol. Reg. Homeost. Agents*, **24**, 307 (2010).
20. Esposito G., Rossi F., Matteini P., Scerrati A., Puca A., Albanese A., Rossi G., Ratto F., Maira G., Pini R. *Laser Surg. Med.*, **45**, 318 (2013).
21. Lauto A., Stoodley M., Marcel H., Avolio A., Sarris M., McKenzie G., Sampson D.D., Foster L.J.R. *Laser Surg. Med.*, **39**, 19 (2007).
22. Lauto A., Hook J., Doran M., Camacho F., Poole-Warren L.A., Avolio A., Foster L.J.R. *Laser Surg. Med.*, **36**, 193 (2005).
23. Ratto F., Matteini P., Centi S., Rossi F., Pini R. *J. Biophotonics*, **4**, 64 (2011).
24. Saxena V., Sadoqi M., Shao J. *J. Pharm. Sci.*, **92**, 2090 (2003).
25. Hu M., Chen J.Y., Li Z.Y., Au L., Hartland G.V., Li X.D., Marquez M., Xia Y.N. *Chem. Soc. Rev.*, **35**, 1084 (2006).
26. Murphy C.J., Gole A.M., Stone J.W., Sisco P.N., Alkilany A.M., Goldsmith E.C., Baxter S.C. *Accounts Chem. Res.*, **41**, 1721 (2008).
27. Huang X.H., Neretina S., El-Sayed M.A. *Adv. Mater.*, **21**, 4880 (2009).
28. Matteini P., Ratto F., Rossi F., Pini R. *J. Biomed. Opt.*, **17**, 0107011 (2012).
29. Jain P.K., Lee K.S., El-Sayed I.H., El-Sayed M.A. *J. Phys. Chem. B*, **110**, 7238 (2006).
30. Mercatelli R., Romano G., Ratto F., Matteini P., Centi S., Cialdai F., Monici M., Pini R., Fusi F. *Appl. Phys. Lett.*, **99**, 131113 (1–3) (2011).
31. Grzelczak M., Perez-Juste J., Mulvaney P., Liz-Marzan L.M. *Chem. Soc. Rev.*, **37**, 1783 (2008).
32. Ratto F., Matteini P., Rossi F., Pini R. *J. Nanopart. Res.*, **12**, 2029 (2010).
33. Ratto F., Matteini P., Cini A., Centi S., Rossi F., Fusi F., Pini R. *J. Nanopart. Res.*, **13**, 4337 (2011).
34. Ratto F., Matteini P., Rossi F., Menabuoni L., Tiwari N., Kulkarni S.K., Pini R. *Nanomed-Nanotechnol.*, **5**, 143 (2009).
35. Alkilany A.M., Murphy C.J. *J. Nanopart. Res.*, **12**, 2313 (2010).
36. Khlebtsov N., Dykman L. *Chem. Soc. Rev.*, **40**, 1647 (2011).
37. Agarwal A., Huang S.W., O'Donnell M., Day K.C., Day M., Kotov N., Ashkenazi S. *J. Appl. Phys.*, **102**, 064701 (2007).
38. Li P.C., Wang C.R.C., Shieh D.B., Wei C.W., Liao C.K., Poe C., Jhan S., Ding A.A., Wu Y.N. *Opt. Express*, **16**, 18605 (2008).
39. Mallidi S., Larson T., Tam J., Joshi P.P., Karpiouk A., Sokolov K., Emelianov S. *Nano Lett.*, **9**, 2825 (2009).
40. Huang X.H., El-Sayed I.H., Qian W., El-Sayed M.A. *J. Am. Chem. Soc.*, **128**, 2115 (2006).
41. Chen J.Y., Wang D.L., Xi J.F., Au L., Siekkinen A., Warsen A., Li Z.Y., Zhang H., Xia Y.N., Li X.D. *Nano Lett.*, **7**, 1318 (2007).
42. Dickerson E.B., Dreaden E.C., Huang X.H., El-Sayed I.H., Chu H.H., Pushpanketh S., McDonald J.F., El-Sayed M.A. *Cancer Lett.*, **269**, 57 (2008).
43. Niidome T., Yamagata M., Okamoto Y., Akiyama Y., Takahashi H., Kawano T., Katayama Y., Niidome Y. *J. Control Release*, **114**, 343 (2006).
44. Prencipe G., Tabakman S.M., Welsher K., Liu Z., Goodwin A.P., Zhang L., Henry J., Dai H.J. *J. Am. Chem. Soc.*, **131**, 4783 (2009).
45. Rayavarapu R.G., Petersen W., Hartsuiker L., Chin P., Janssen H., van Leeuwen F.W.B., Otto C., Manohar S., van Leeuwen T.G. *Nanotechnology*, **21**, 145101 (2010).
46. El-Sayed I.H., Huang X.H., El-Sayed M.A. *Nano Lett.*, **5**, 829 (2005).
47. Loo C., Lowery A., Halas N.J., West J., Drezek R. *Nano Lett.*, **5**, 709 (2005).
48. Huff T.B., Tong L., Zhao Y., Hansen M.N., Cheng J.X., Wei A. *Nanomedicine-UK*, **2**, 125 (2007).
49. Shi X.G., Wang S.H., Meshinchi S., Van Antwerp M.E., Bi X.D., Lee I.H., Baker J.R. *Small*, **3**, 1245 (2007).

50. Tong L., Wei Q.S., Wei A., Cheng J.X. *Photochem. Photobiol.*, **85**, 21 (2009).
51. Lu W., Zhang G.D., Zhang R., Flores L.G., Huang Q., Gelovani J.G., Li C. *Cancer Res.*, **70**, 3177 (2010).
52. Stanwix H. *Nanomedicine-UK*, **8**, 10 (2013).
53. Lapotko D.O., Lukianova E., Oraevsky A.A. *Laser Surg. Med.*, **38**, 631 (2006).
54. Nikoobakht B., El-Sayed M.A. *Langmuir*, **17**, 6368 (2001).
55. Daniel M.C., Astruc D. *Chem. Rev.*, **104**, 293 (2004).
56. Nikoobakht B., El-Sayed M.A. *Chem. Mater.*, **15**, 1957 (2003).
57. Gou L.F., Murphy C.J. *Chem. Mater.*, **17**, 3668 (2005).
58. Pini R., Rossi F., Menabuoni L., Lenzetti I., Yoo S., Parel J.M. *Ophthal Surg. Las. Im.*, **39**, 260 (2008).
59. Mercatelli R., Ratto F., Centi S., Soria S., Romano G., Matteini P., Quercioli F., Pini R., Fusi F. *Nanoscale*, **5**, 9645 (2013).
60. Ungureanu C., Kroes R., Petersen W., Groothuis T.A.M., Ungureanu F., Janssen H., van Leeuwen F.W.B., Kooyman R.P.H., Manohar S., van Leeuwen T.G. *Nano Lett.*, **11**, 1887 (2011).
61. Joshi P.P., Yoon S.J., Hardin W.G., Emelianov S., Sokolov K.V. *Bioconjugate Chem.*, **24**, 878 (2013).
62. Matteini P., Ratto F., Rossi F., Rossi G., Esposito G., Puca A., Albanese A., Maira G., Pini R. *J. Biomed. Opt.*, **15**, 041508 (2010).
63. Matteini P., Dei L.G., Carretti E., Volp N., Goti A., Pini R. *Biomacromolecules*, **10**, 1516 (2009).
64. Esposito G., Rossi F., Matteini P., Ratto F., Sabatino G., Puca A., Albanese A., Rossi G., Marchese E., Maira G., Pini R. *J. Biol. Regul. Homeost. Agents*, **26**, 447 (2012).
65. Matteini P., Ratto F., Rossi F., Centi S., Dei L., Pini R. *Adv. Mater.*, **22**, 4313 (2010).
66. Matteini P., Ratto F., Rossi F., de Angelis M., Cavigli L., Pini R. *J. Biophotonics*, **5**, 868 (2012).
67. Matteini P., Martina M.R., Giambastiani G., Tatini F., Cascella R., Ratto F., Cecchi C., Caminati G., Dei L., Pini R. *J. Mater. Chem. B*, **1**, 1096 (2013).
68. Matteini P., Tatini F., Luconi L., Ratto F., Rossi F., Giambastiani G., Pini R. *Angew. Chem. Int. Edit.*, **52**, 5956 (2013).
69. Stuart M.A.C., Huck W.T.S., Genzer J., Muller M., Ober C., Stamm M., Sukhorukov G.B., Szleifer I., Tsukruk V.V., Urban M., Winnik F., Zauscher S., Luzinov I., Minko S. *Nat. Mater.*, **9**, 101 (2010).
70. Zhou S.B., Deng X.M., Yang H. *Biomaterials*, **24**, 3563 (2003).