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Unbreakable Codes in Electrospun Fibers: Digitally Encoded Polymers to Stop Medicine Counterfeiting

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Health organizations claim that there should be 'zero tolerance' for counterfeit medicines, yet the problem is growing worldwide[1-10]. Labelling the tablet itself ("one-dose marking"), instead of package labelling, could be a powerful strategy to protect patients from fake drugs. Unfortunately, these methods are not in use today. This report proposes one viable solution: digitally encode polymers, which already form a major class of pharmaceutical excipients, and place them within tablets. Fluorescent polymer solutions can easily be electrospun into micron sized fibers and subsequently encoded by a photobleaching process. We have shown that, provided the physicochemical interactions between the fluorophore and the polymer are favourable, amorphous polymer fibers can be encoded with long lasting digital codes. Encoded fibers at the surface of tablets can be quickly decoded by using a basic fluorescence microscope. The materials and strategy used in this concept are simple and cheap, making it of special importance to patients in developing countries who now suffer or even die due to counterfeited medicines of inferior quality.

Official health institutes recognize that drug counterfeiting is an ever increasing hazard to unaware consumers[1-10]. Especially considering the rising popularity of purchasing drugs via the internet, counterfeit drugs are becoming widespread[11, 12]. In industrialized countries, particularly today's "lifestyle drugs" (e.g. sexual performance enhancers, smoking cessation agents) are targeted for easy profit. However, in developing countries antibiotics, painkillers, anti-malarial and HIV drugs are also being counterfeited. All too often, such counterfeits have caused patients' deaths.

To combat counterfeiting, the packaging of an increasing number of drugs is being 'protected' by radio frequency tags, barcodes, watermarks, fluorescent inks, chemical or biological (DNA) tags[13]. Unfortunately, such tracking technologies are only effective if the drugs are not repackaged. Manufacturers often do not ship drugs directly to hospitals and dispensing pharmacies. Generally, drugs are sold to Wholesalers or Distributors who repackage drugs from bulk to unit-of-use containers which provide a means for counterfeit drugs to enter the legitimate drug supply chain. An authentic package does *not* therefore, certify authentic content. To overcome this, "in-drug labelling" itself,

instead of on the drug packaging, could help defeat drug counterfeiters. Nowadays, the incorporation of taggants (like color-coded particles and mica particles coated with the colorants titanium oxide and/or iron oxide) in drug formulations is seldom used; one major reason being that it requires extensive toxicological screening of the taggant and formulation compatibility testing.

Tablets are the most widely used drug dosage form in the world. Recently we introduced digitally encoded polystyrene microparticles (named 'memobeads') for the in-product labeling of tablets[14-17]; information is written in the middle plane of fluorescently dyed microspheres by 'spatial selective photobleaching' of the fluorescence by the use of a confocal laser scanning microscope. 'On-tablet laser NanoEncryption' was also announced, to write digital codes on the surface of tablets[18]. There has been also an interest in Raman spectroscopy to analyse the composition of the pharmaceutical excipients of a tablet[19]. Indeed, such an "excipient-fingerprint" could also be a tool to track down counterfeiters. Clearly, the future of Raman imaging of pharmaceutical excipients will need skilled personnel and expensive instruments, meanwhile, developing countries continue to suffer severely from counterfeited drugs.

We took up this challenge; to "digitally encode polymers", being the most widely used class of excipients in oral medicine, tablets, today. Our objective was to digitally encode polymers that are FDA approved for use in oral medicines, aiming to use them in trace amounts. Obviously, after encoding the polymers must remain harmless and decoding should occur by a fast, low-cost technique. This paper launches the concept of encoding a polymer by mixing the polymer solution with a fluorophore, electrospinning the solution into micron sized fibers and encoding the fibers by photobleaching. Two arguments explain our choice for electrospinning. Firstly, it is a remarkably simple technique that allows preparing the most simple polymeric matrices from a wealth of pharmaceutical polymers. Secondly, electrospinning allows aligning the microfibers which is a requirement when encoding the fibers by a scanning laser beam that locally destroys the fluorophores. Our reason for encoding the fibers *by photobleaching* was the observation that many polymers used in tablets form amorphous matrices with a rather high glass transition temperature (Tg), a request to get fluorophores sufficiently immobilized in the microfibers at room temperature. Keep in mind, mobile fluorophores would result in a recovery of the fluorescence in the bleached zones and thus a disappearance of the code.

We made use of a rotating wheel equipped with a glass support to collect the fluorescent fibers during the electrospinning process (Figure 1A). Highly aligned polystyrene (PS), ethylcellulose (EC), cellulose-acetate-phthalate (CAP), and poly(lactic-co-glycolic acid) (PLGA)-microfibers could be obtained by rotating the wheel at high speed. As Figure 2A illustrates, the higher the rotating speed, the better the fibers were aligned.

Finding an optimal fluorophore was a challenge, as the dye should fulfill numerous requirements. a) It should sufficiently dissolve and be stable in the organic solvent(s) used to solve the

polymers. b) It should become sufficiently and homogenously encapsulated in the fibers. c) It should be strongly immobilized in the polymer matrix thereby obtaining a long-lasting code and to avoid losing dye into the water wherein the fiber pieces are dispersed to apply them to the tablets. d) The dye should bleach out from the intense laser beam of the encoding device. Finally, the fluorophore should be allowed to use in oral drugs.

Fluorescein is a common water-soluble fluorophore. Fluorescein capsules are taken by patients to diagnose ophthalmologic diseases[20, 21]. We loaded the fibers with the fluorescein derivative fluoresceinisothiocyanate (FITC), which is soluble in DMF and THF used to solve PS, EC, CAP and PLGA. Figure 2B1 shows that PS-fibers can be homogeneously loaded with FITC though, one day after keeping the fibers in open air at room temperature, FITC seems to have significantly migrated towards the surface of the fibers (Figure 2B2). And especially upon dispersion in water, FITC significantly leaks from the fibers into the water (Figure 2B3). An insufficient immobilization of FITC in the PS-fiber and a too high solubility in water made FITC unsuitable. We continued with coumarin-6. As Figures 2C2 and 2C3 show, even after 4 days' storage in open air and after one day being dispersed in water, PS-fibers loaded with coumarin-6 remained homogeneously coloured. This was not only the case for PS-fibers, coumarin-6 seemed also well suited to design stable homogeneously coloured EC-, CAP- and PLGA-fibers (Figure 3 row A).

The fabrication of small fiber pieces has recently received attention. In one method fiber pieces have been fabricated by the use of templates[21, 22]; in another study (long) fibers made by electrospinning were removed from the drum and cut by razor blades under liquid nitrogen into 50-100µm rod-like pieces[23]. As Figure 3 panel B shows, a scanning UV laser could cut the aligned PS-, EC-, CAP- and PLGA-fibers into 100-200 µm long pieces. Figure 3 panel C shows that the coumarin-6 loaded fiber pieces could be digitally encoded by photobleaching by a laser beam scanning along the longitudinal axis of the fiber pieces (Figure 1D). The encoded fiber pieces were named 'memofibers'.

For the purpose of this study a stable code is an absolute requirement. Clearly, the more mobile the fluorophore in the polymer fibers, the faster the digital code will disappear. If one assumed coumarin-6 diffusing in water, one could easily calculate from Stokes-Einstein law that it would take only seconds to travel a few micrometers. As the encoded segments are typically five to ten micrometers, it is a challenge to sufficiently immobilize the fluorophore in the fibers to avoid even the smallest displacement over the long term (months, years) the codes are expected to survive.

Figure 3 panel E shows the contrast of the codes as a function of time. Clearly, in EC-fibers the code fades away over time. Although, in PS-, CAP- and PLGA-fibers the fluorescence recovery in the bleached segments is very weak keeping the codes clearly legible after 4 months' storage. The reason the code in the EC-fibers disappears remains unknown. Calorimetric measurements revealed that the fibers showed a Tg of around 95 (PS) 133 (EC), 160 to 170 (CAP) and 50 °C (PLGA). All fibers were thus in amorphous state at room temperature. Very likely, a lower affinity between the fluorophore and the EC-matrix explains the fading of the codes in the EC-fibers.

In the next step we evaluated whether the codes in fiber pieces deposited at the surface of tablets could be identified. Figure 3 panel D shows the fluorescence images we obtained from fibers at the surface of tablets using a simple fluorescence microscope. The digital codes are perfectly readable. Even after subjecting the tablets to a friability test the codes remained readable indicating that shear stresses do not detach memofibers from the tablets.

The concept of digitally encoding polymers by just mixing them with a fluorophore and spinning into simple fibers, then simply encoding them by spatial photobleaching, is especially attractive as it is broadly applicable to the wealth of polymers being used. With respect to the digital encoding of tablets, one could raise the question why memofibers should be preferred over memobeads which we introduced before[14, 17]. **a)** In memobeads the code is written in the central plane of polystyrene microspheres, requiring a proper orientation of the spheres for decoding. Therefore memobeads have to remain magnetic by adding ferromagnetic chromium dioxide particles. Due to the cylindrical geometry of the polymer fibers a proper orientation of the fibers is not necessary for decoding; regardless of the position of the fibers the codes are readable. **b)** Memofibers are simply composed of the pharmaceutical ingredient (i.e. the polymer) and a fluorophore while memobeads contain many more types of materials. Logically, the simpler the chemical composition, the easier it is to get the materials approved for use in medicines. We estimate that a 100 µm memofiber contains as little as 0.1 picogram coumarin-6; If we apply 10 fibers per tablet and assume a patient would take 10 tablets a day, the daily intake of coumarin-6 would be around 10 picogram. An "impurity" in a drug formulation is allowed as long as the daily intake remains lower than 1.5 microgram[24]. **c**) Memofibers can be made from various types of pharmaceutical polymers, which is attractive considering the various polymers used in oral medicines. Designing memobeads from polymers other than polystyrene would be a huge challenge, to say the least. **d**) As both the length and the thickness of memofibers can be easily controlled through appropriate spinning and cutting, it is very possible to store information in the length as well as the diameter of the memofibers. Changing the diameter of microspheres, and especially, making monodispers microspheres, remains a big technical challenge. Some other types of encoded cylindrically shaped geometries (rods) have been reported as well, however, those are all metal based (Al, Ni, Pd) rods[25, 26], which are not suited for the labeling of medicines. **e**) To decode memobeads they should be recovered from the tablets, whereupon one needs an expensive confocal fluorescence microscope and a magnetic field to read the code. Luckily, memofibers can be directly decoded at the tablet's surface by a much cheaper fluorescence microscope.

As Figure 4 shows, digital codes can be written in the polymer fibers of woven scaffolds too which are under investigation as e.g cell carriers for tissue engineering[27]. In such an "encoded web" the codes remain at fixed positions in space, thus allowing to identify positions in a three dimensional environment. The encoded-fiber-concept thus shows potential beyond the protection of pharmaceuticals against counterfeiting. As an example, one can imagine the loading of such webs with

different types of cells (or different types of bacteria) whereby the codes in the fibers allow to remember which type of cells (or bacteria) is present at a specific location. As another example, fibers in such a web may be loaded with different types of bioactive compounds (like e.g. each fiber loaded with another growth factor) to be locally released to cells which are supported by the web: the codes in the fiber may allow to identify to which types of drugs cells in specific areas of a web were exposed.

In summary, this manuscript proposes to digitally encode oral medicines, in particular tablets containing drugs, using micron sized fibers made from pharmaceutical polymers, which are already a major class of excipients in oral drugs. We've shown that such memofibers can be readily obtained by electrospinning the polymer (PS, CAP and PLGA in this study, the two latter ones being frequently used in tablets[28] and subsequently cutting the long, aligned fibers into micrometer sized pieces using an appropriate scanning UV laser. Key to this concept is the immobilization of a fluorophore in glassy polymer fibers. Provided the glass transition temperature of the polymer fiber and the physicochemical interactions between the fluorophore and the polymer are favourable, we showed that the barcodes written in the fibers through photobleaching are stable over long periods. We've also provided evidence that memofibers at the tablet's surface can be easily decoded using a basic fluorescence microscope, even without removing the fiber pieces from the tablets. This simple, inexpensive strategy makes the 'digital encoded polymer fiber' even more attractive for use in developing countries, especially where patients suffer and die from counterfeited medicines of inferior quality. Nowadays "one-dose marking" is, to our knowledge, not in use; the major reasons being that a) only very recently have some technologies been proposed[14, 17, 18] and b) extensive toxicological screening and expensive formulation compatibility testing are needed in cases where taggants are added to these medicines. In our opinion, though highly advanced in use, the simple memofibers we presented will initiate the much needed solution to the in-product labelling of pharmaceuticals, especially as memofibers are made of those pharmaceutical ingredients having already been used for decades in tablets and other types of oral medicines.

Methods

Microfibers were obtained by electrospinning polymer solutions containing a fluorophore (see Table 1 in *Supporting Information*). An electrospinning setup (Figure 1A) requires a power supply, a syringe, a flat tip needle and a grounded collector[29]. A droplet of the polymer/fluorophore solution is formed at the tip of the needle by surface tension while charge is induced on the droplet surface by an electric field. When the electric field reaches a critical value at which the electric force overcomes the surface tension of the droplet, a charged jet is ejected from the tip. While the jet travels in air, the solvent evaporates, resulting in the deposition of fibers on a collector. We used a rotating collector fitted with a glass support (Figure 1B) to collect aligned fibers.26 The fibers on the glass support were dried in

open air. As shown in Figure 1C, the aligned fluorescent fibers were cut into small pieces by cold ablation using a PALM MicroBeam System Version 4.0 AxioVert laser equipped with a 355 nm pulsed UV-Laser[30].

The fluorescent fiber pieces were encoded by spatial selective photobleaching by exposing well selected regions to a 488 nm laser beam (Figure 1D). An in-house-developed encoding device was used, being a laser scanning confocal microscope (Nikon) equipped with an argon laser and an acousto-optic modulator[15, 16, 31, 32]. Upon bleaching, the fluorescent molecules lose their fluorescence, giving rise to the code. The encoded fiber pieces (named 'memofibers') were dispersed in water, simply by applying a drop of water on the fiber pieces on the glass support.

Using a lactose/microcrystalline cellulose mixture 20 mm tablets were prepared. 2µl of a memofiber dispersion were dripped onto the surface of the tables (Figure 1E) and subsequently dried at 37°C for 12h. For decoding, the surface of the tablets was imaged by a (non-confocal) fluorescence microscope.

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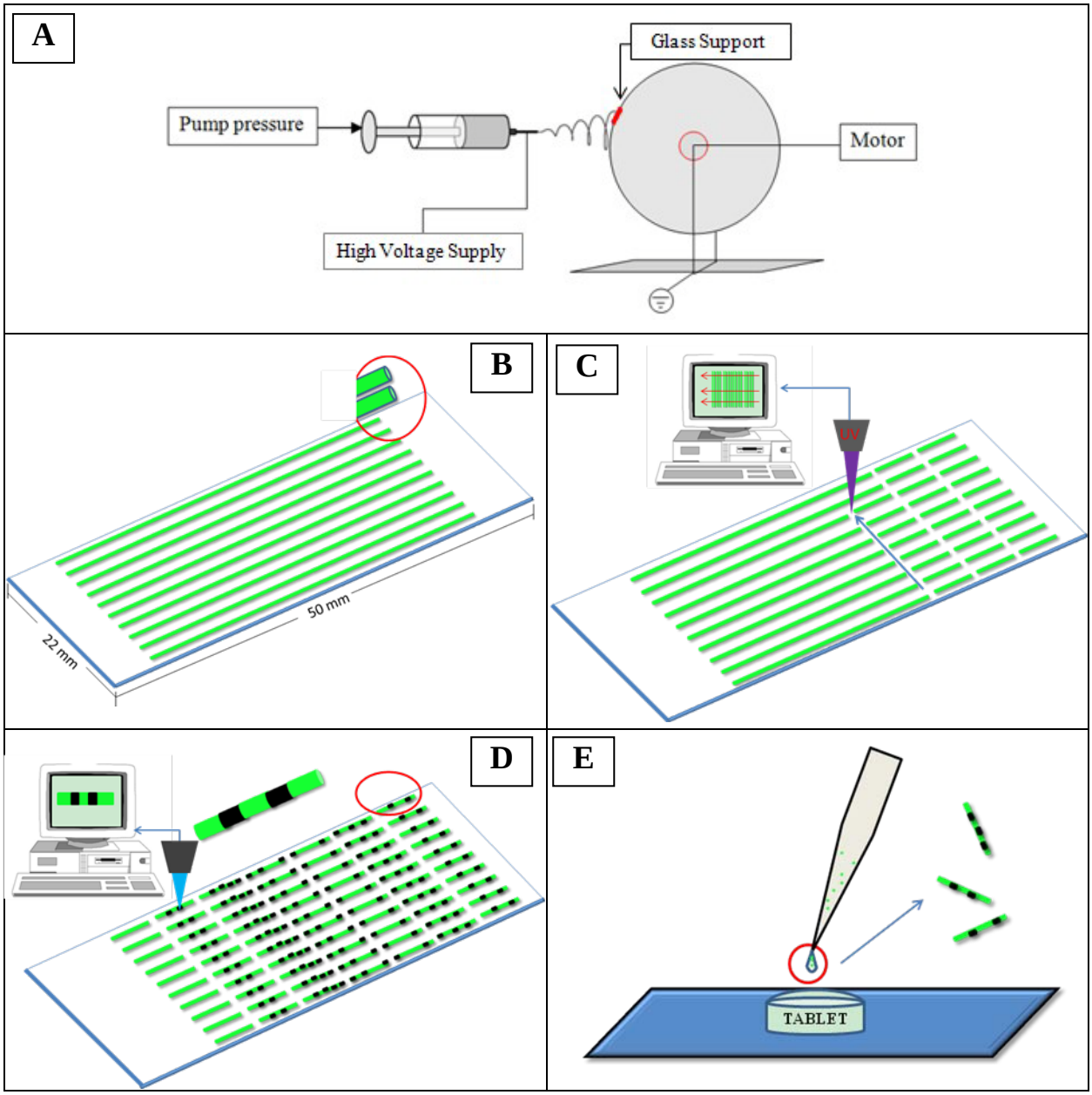

Figure 1. Schematic representation of the synthesis of memofibers. **A)** Electrospinning setup. **B)** Aligned polymer fibers deposited by electrospinning on the glass support. **C)** Cutting of the polymer fibers into fiber pieces by cold ablation. **D)** Encoding of the fiber pieces by photobleaching through the use of a scanning laser beam. **E)** Applying a few microliters of a memofiber dispersion on the surface of a tablet.

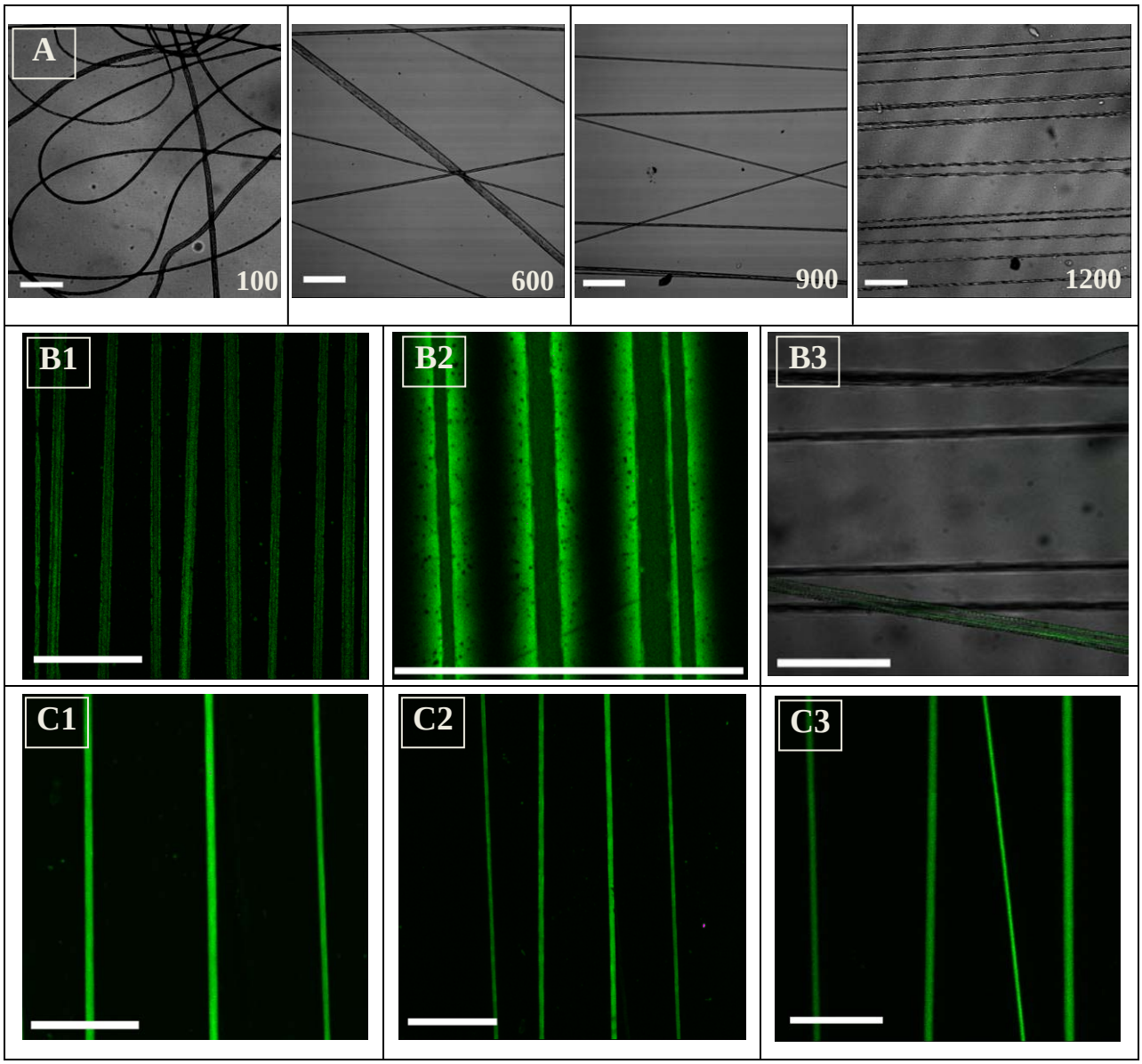

Figure 2. Aligned fluorescent polymer fibers **A)** Transmission image of PS-fibers collected at different speeds of the rotating wheel (100 to 1200 rpm). B) Fluorescence images of aligned PS-fibers loaded with FITC: B1 - immediately after spinning, B2 - 1 day after keeping the fibers in open air at room temperature, B3 - 22 hours after dispersing the fibers in water; FITC significantly leaks from the fiber into the water (B3 is a combined transmission/fluorescence image as the fibers were no longer sufficiently fluorescent). **C**) same as for B but it concerns aligned PS-fibers loaded with coumarin-6. Scale bar is 100μm.

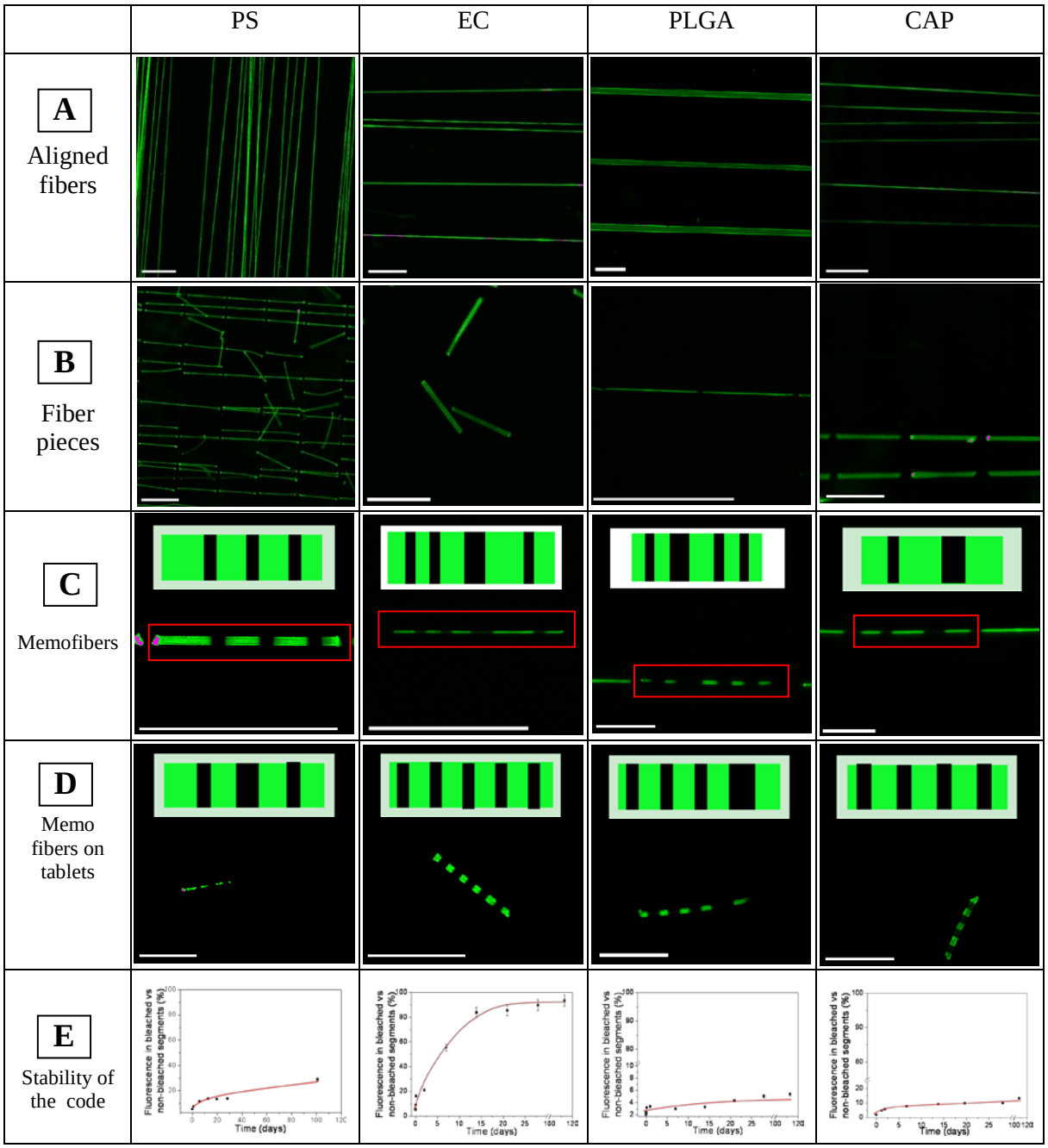

Figure 3. Fluorescence images of aligned fibers (**A**) and fiber pieces (**B,C,D**) loaded with coumarin-6. Note that information can be stored , not only in the width of the bars, but as well in the length of the memofibers. The inserts in **C** and **D** show the barcodes which were written in the fiber pieces, the red rectangles indicate one memofiber. **E**) shows the contrast of the digital codes in the fiber pieces kept in open air at room temperature. The contrast of the code is defined as the ratio (%) of fluorescence in the bleached segments to the fluorescence in the non-bleached regions in the fiber. Scale bar is 100µm.

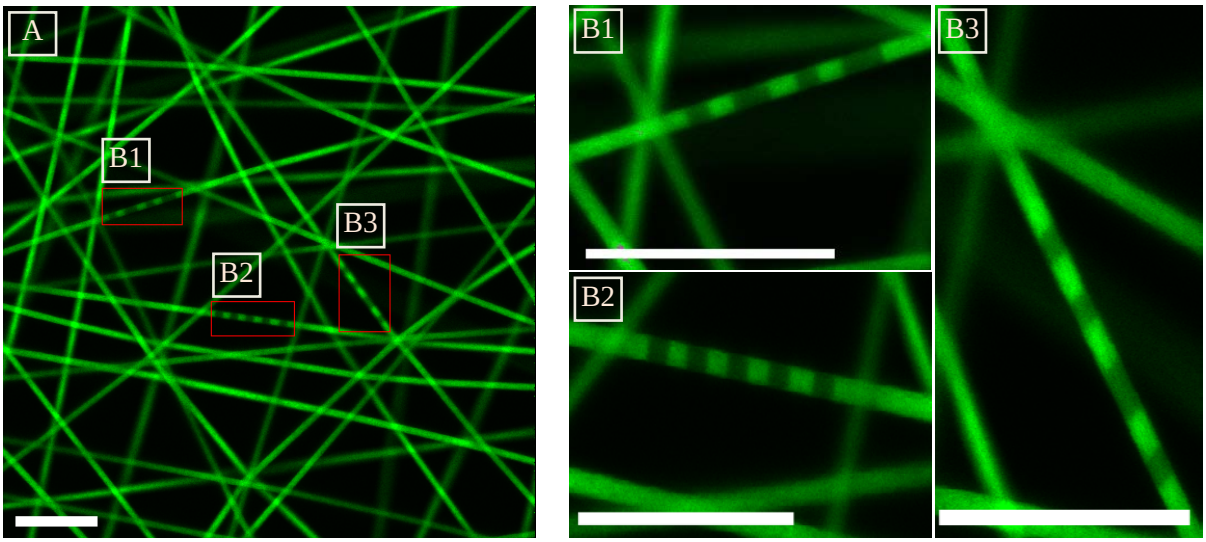

Figure 4. Digitally encoded polymer webs. Fluorescence images of non-aligned PS-fibers loaded with coumarin-6 and encoded by photobleaching. The middle and right panel are close-ups of the digital codes in the web. The scale bar is 50μm.