Universal Strategy for Designing Shape Memory Hydrogels

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ABSTRACT: Smart polymeric biomaterials have been the focus of many recent biomedical studies, especially those with adaptability to defects and potential to be implanted in the human body. Herein, we report a versatile and straightforward method to convert non-thermoresponsive hydrogels into thermoresponsive systems with shape memory ability. As a proof-of-concept, a thermoresponsive polyurethane mesh was embedded within a methacrylated chitosan (CHTMA), gelatin (GELMA), laminarin (LAMMA) or hyaluronic acid (HAMMA) hydrogel networks which allowed to obtain hydrogel composites with shape memory ability. With this system, we achieved good to excellent shape fixity ratios (50-90%) and excellent shape recovery ratios (~ 100%, almost instantaneously) at body temperature (37 °C). Cytocompatibility tests demonstrated good viability either with cells on top or encapsulated during all shape memory processes. This straightforward approach opens a broad range of possibilities to convey shape memory properties to, virtually, any synthetic or natural-based hydrogel for several biological and non-biological applications.

Researchers have turned their attention to smart polymeric biomaterials and its potential use in medical devices, including structures for tissue engineering and regenerative medicine (TERM)[1]. Particularly, shape memory polymers (SMP), which can undergo a predefined shape change in response to a given stimulus[2, 3], have been explored. The ability of these materials to adapt to specific defect sites and potentially be implanted through minimally invasive strategies[4], presents a considerable advantage for the development of advanced biomedical devices[5,6,7]. Whenever possible, the use of natural polymers as building blocks to produce such materials allows taking advantage of its most beneficial properties, namely biocompatibility, biodegradability, and ability to promote biological activity[8, 9]. However, most natural polymers do not possess stimuli-responsive features to shape memory capability in physiological conditions, even after performing chemical modifications[10]. This drawback has been largely surpassed by producing hybrid materials[11], in which natural polymers are combined with synthetic ones to integrate both the biocompatibility properties of the first and the stimuli-responsive behavior of the latter.

Different stimuli can be employed to trigger shape memory behavior (e.g., light, pH, humidity, among others)[9, 12]. However, temperature-sensitive SMPs have attracted significant interest over a wide range of applications.[13] Among the synthetic temperature-responsive polymers available for biomedical applications, polyurethanes have stood out due to their biocompatible and biodegradable character, as well as their tunable and favorable mechanical properties[14,15]. In addition, the transition temperature (T_{trans}) of polyurethane (~32 °C) is close to human body temperature[16] unlike other temperature-responsive polymers.[13] Recently, Hendrikson *et al.*[17] employed a shape polyurethane (SMPU) network to build scaffolds with 4D functionality, i.e., which change their shape when exposed to a given stimulus. The authors tested the temperature-induced shape memory ability of the developed scaffolds and evaluated how this process impacted cells seeded onto this material, concluding that the shape recovery process influenced cell morphology[17].

Inspired by this study, we conjectured whether this SMPU network could be used to impart shape memory ability onto any hydrogel, particularly when the hydrogel precursors do not possess stimuli responsiveness properties. We hypothesized that by embedding a SMPU structure, for example in the form of a mesh, within a non-thermoresponsive hydrogel network, the SMPU would allow the surrounding hydrogel to follow its movement upon shape fixity and recovery, conveying the shape memory property to the overall composite (Figure 1). To the best of our knowledge, this is a new approach for designing shape memory hydrogels with an universal tone, broadening the spectrum of polymers employed to build shape memory biomaterials. In this sense, to demonstrate the concept, we used methacrylated polymers such as methacrylated chitosan (CHTMA), laminarin (LAMMA) and hyaluronic acid (HAMMA), which do not show temperature-induced shape memory behavior as precursor biomaterials for the hydrogel component. We also evaluated the temperature-responsive methacrylated gelatin (GELMA) performance in this system.

These polymers were chosen as simple and widely known platforms with described cytocompatibility [18-22] (Figure 1).

With this, we propose a simple and broad range strategy

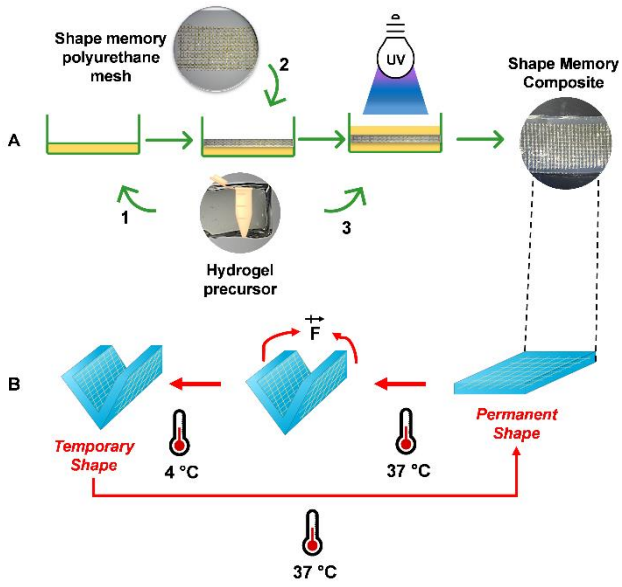

Figure 1. Schematic representation of the (A) Preparation of Polymer-SMPU composite and its (B) Shape memory behavior.

for fabricating a composite capable of adapting to a specific defect, including hydrogels populated with cells either by encapsulation or cell seeding process. Initially, we prepared the non-thermoresponsive natural derived material. For that, CHTMA, GELMA, LAMMA and HAMMA were synthetized by coupling chitosan (CHT), gelatin (GEL), laminarin (LAM) or hyaluronic acid (HA) with methacrylic acid, methacrylic anhydride or glycidyl methacrylate-based on previous procedures[18, 21-23]. CHTMA, GELMA, LAMMA and HAMMA were obtained as white solids with a substitution degree (DS) of 33%, 52%, 18% and 11% (and 29%), respectively. The insertion of methacrylate moieties onto the polymers backbones was confirmed and quantified by ^{1}H nuclear magnetic resonance (NMR) or ultraviolet-visible (UV-Vis) spectroscopy analysis (Figure S1-S5). The ^{1}H NMR spectra showed two singlets around 6.00 ppm, corresponding to the characteristic hydrogens H_a and H_b of the inserted methacrylic moieties and a singlet around 2.00 ppm, corresponding to the methyl group hydrogens H_e of the inserted methacrylate moiety, which was in good agreement with already reported data[21-23]

SMPU meshes with a square pore size of 500 µm were fabricated via melt electrowriting technique from SMPU pellets of Mw=77 800 Da, Mn=43 000 Da, PDI=1.80 a glass transition temperature of 35 °C (according to SMP Technologies datasheet). The fiber diameter of the SMPU meshes was 58.6 ± 3.7 µm, resulting in pores of 456.3 ± 23 µm in size.

To impart the hydrogel with shape memory capability, we envisioned a general pathway depicted in Figures 1, 2A & S6. A CHTMA, GELMA, LAMMA or HAMMA hydrogel strip [(5% w/v solution in cell culture medium (α-MEM)] was covalently photocrosslinked (by exposure to UV light) with an embedded SMPU mesh (19.0 mm x 4.0 mm x 0.55 mm) in its network, corresponding to the initial/permanent shape of the polymer-SMPU composite. Composites were then exposed to a 65 °C or 37 °C to mold better and deform the SMPU mesh, and a temporary "U" or "L" shape was created by applying an external force, followed by the fixation process at 4 °C for 6 h (Figure 2A & S6). The shape fixity ratio (R_f) was then analyzed to determine the ability to fix the desired temporary form (Figure 2A). Subsequently, the sample was placed in a pre-heated (37 °C) cell culture medium to promote the recovery to the permanent shape (Figure 2A & S6) and assess its shape recovery ratio (R_r), to determine the ability of this composite to recover to its original (permanent) form (Figure 2A & S6).

We decided to evaluate parameters for each pathway step of our shape memory process. For the deformation step, we started our study by testing 65 °C (the same temperature used by Hendrikson *et al.*[17]) and then 37 °C, using polymer-SMPU samples (20.0 mm x 5.0 mm x 3.0 mm) with different UV light exposure periods (90 s and 120s). The temperature (65 °C and 37 °C) did not affect the R_f results (data not shown). So, we chose to move forward with 37 °C to recapitulate the physiological conditions better. The obtained R_f for all conditions, under 37 °C, are compared in Figure 2. The R_f of CHTMA-, GELMA- and HAMA-SMPU composites was not significantly affected by either UV light exposure periods. This could indicate an almost complete crosslink after 90 s exposure. However, 90s of UV light exposure was not enough for efficient crosslinking of the LAMMA network, maybe because of its low molecular weight compared to the other polymers. We observed 50-80% R_f range, with HAMA-SMPU achieving the highest R_f, ~80%, and CHTMA-SMPU composite showing the lowest result, R_f ~50%. We also observed a 5-30% increasing R_f for the polymer-SMPU composites compared to pristine SMPU.

The R_f appears to depend on the hydrogel precursor stiffness, as shown in Figure 3 for the HAMA-SMPU composite. R_f value tends to decrease with the increase of the DS (from11 to 29%) and concentration [5 to 10% (w/v)] of the HAMA. This effect was greater for the DS parameter, resulting in a 20%. R_f decrease.

Another important parameter that could influence the R_f value is the bending angle of the deformation step. For example, the smaller is the angle between the ends of the composite strip (θ_{def}, Table S1, Supporting Information), the greater the force applied will be, which will reach the limit of the material. Our data support this fact, when we increased the deformation angle from ~20° (Figure 2) to 90° (Figure 4) we achieved ~90% R_f, which is an excellent result comparing to other studies[24].

Methacrylated polymers hydrogels themselves displayed a 0% R_f (hydrogels broke into pieces at the deformation step) under all conditions, meaning that the SMPU mesh was responsible for the temperature-induced shape memory behavior of the material. The overall flexibility of the hydrogel matrix permits the deformation controlled by the embedded SPUM mesh. The composites can retain the shape memory behavior of the thin, pristine SMPU mesh.

Concerning the shape recovery step (*i.e.,* returning to the permanent shape), we observed a similar behavior, under all conditions, consistently achieving ~100% R_r after less than 2 min at 37 °C (Figure 2B-ii and Video S1). The results support the system universality and clearly demonstrate that the hydrogels could fully accompany the shape variation of SMPU mesh. So, the mechanism for our shape memory system is based on the well-studied thermoresponsive SMPUs mechanism[3, 25-27]. Their structure composed by alternatively disposition of hard (diisocyanate

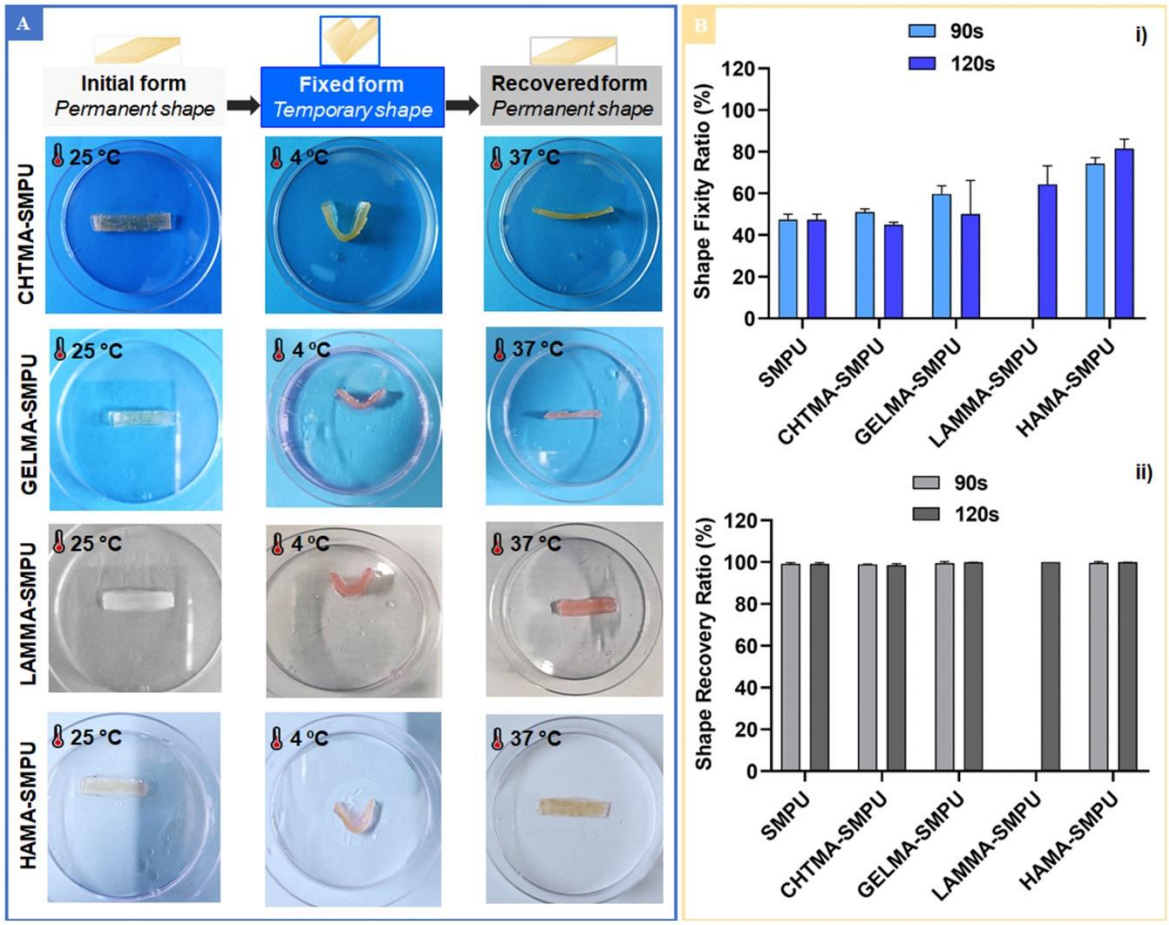

Figure 2. (A) Showcase each stage of the shape memory process of CHTMA-, GELMA-, LAMMA- and HAMMA-SMPU composites. (B) Comparison of SMPU and CHTMA-, GELMA-, LAMMA- and HAMMA-SMPU (with two different reticulation periods: 90 and 120 s) i) shape fixity ratio (%) after a U-shape deformation and ii) shape recovery ratio (%) at 37 °C.

moieties) and soft (butanediol moieties) segments separated by urethane bond allow them to switch between shapes due to the reversible motion between these segments, by working below and above their transition temperature (T_{trans}), 32 °C in our case[17, 26, 27].

Targeting a material capable of adapting to a more realistic defect and mechanically fixing to a cylindrical object, we conducted a shape memory study using a ring shape composite based on CHTMA-SMPU (Figure 5A). As can be observed, we were able to increase the diameter of the composite ring from 5 mm (permanent) to 7 mm (temporary) using an iron tube to apply an external force during the fixation step. Concerning the recovering step, after a few seconds, at 37 °C, we observed the complete recovery to the initial diameter (Figure 5A).

All the polymers explored here have reported good cytocompatibility in many studies. We intend to investigate if the introduction of SMPU could elicit some cytotoxicity. Aiming to prove that the developed system gathered the necessary features for the successful implantation, we evaluated the cytocompatibility for the case of CHTMA-SMPU composite using MC3T3-E1 pre-osteoblasts as a cell model. Based on the beforementioned R_f results and envisioning cytocompatible conditions, we chose to conduct these studies under 37 °C. As depicted in Figure 5B-i and ii, pre-osteoblast cells seeded on top of the CHTMA-SMPU composite remained viable (evidenced by the green staining) and were able to spread throughout the surface. This behavior indicates that the entire process to which the composite was submitted had no impact on the already known cytocompatibility of CHTMA[20]. The possibility for cell encapsulation was assessed by their entrapment in the CHTMA matrix alongside the SMPU mesh (Figure 5B-iii) and subjected to the previously described shape fixation process, using 37 °C as deformation temperature. Encapsulated cells remained viable 1 day after encapsulation (Figure 5B-iv), showing endurance to the entire shape memory process.

A previous report validated the cytocompatibility nature of the SMPU mesh alone, requiring a collagen coating of the SMPU surface for successful cells adhesion[17]. In our case, the inclusion of the CHTMA hydrogel allowed us to suppress that requirement, enabling the creation of a symbiotic composite combining good cytocompatibility and shape memory behavior.

Despite the fixation rate still needing some improvement, with these results, we envisioned that this system could potentially be applied in several fields such as biomedical devices for TERM (e.g., smart valves[28], bone[29], cartilage[30], neural regeneration[31] and cardiac patches[32] or vascular stents[33]), drug delivery[34], softs robotics/actuators and 3D/4D (bio)printing[3, 9, 11].

In summary, a novel and versatile approach to convey shape memory properties to virtually any synthetic or natural-based hydrogel was achieved, opening new

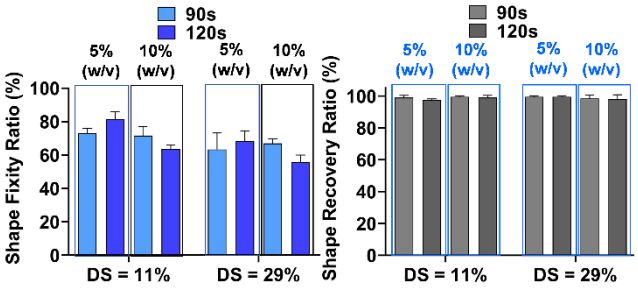

Figure 3. Degree of substitution (DS) and concentration (%w/v) effect in shape fixity ratio (left side) and shape recovery ratio (right side) for HAMA-SMPU composite (with two different reticulation periods: 90 and 120 s).

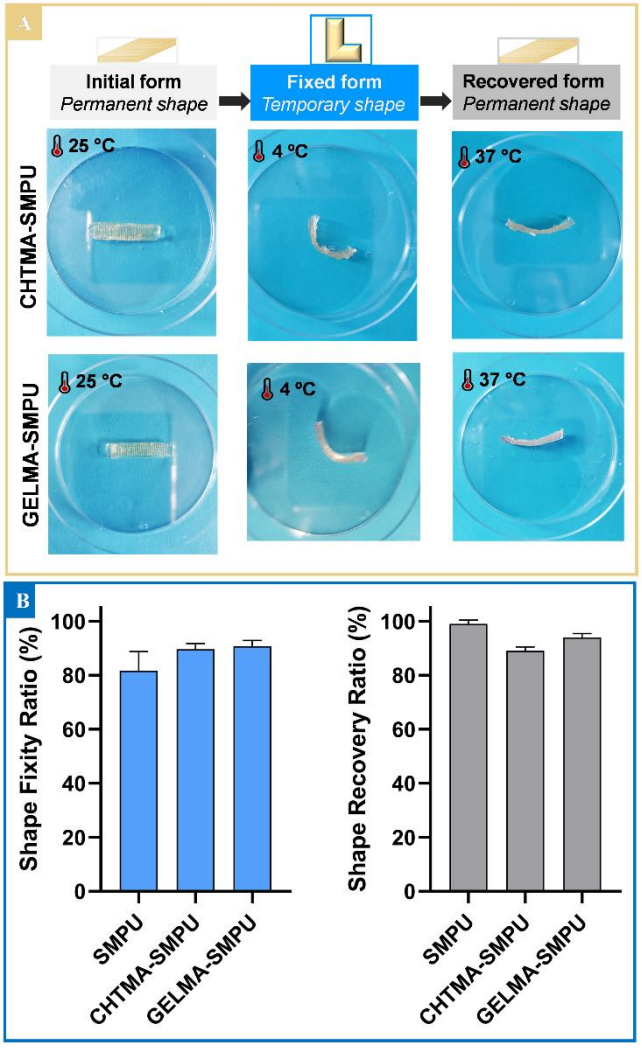

Figure 4. (A) Digital pictures of each stage of the shape memory process for a temporary L-shape. (B) Shape fixity ratio (%) and shape recovery ratio (%) for CHTMA-SPMU and GELMA-SMPU composites.

to biomaterials that intrinsically do not have this ability. On the other hand, this strategy may also be used to bestow temperature responsiveness onto a hydrogel that already displays responsive behavior to other stimuli (pH, light, among others), conveying multiple shape changes on a single material, triggered by different stimuli. The shape memory element introduced in the hydrogel was a mesh prepared by melt electrowriting, but we could envisage structures with more complex architectures that could be assembled with various types of hydrogels. The cytocompatibility test also supports that this system can be used as a medical device in TERM.

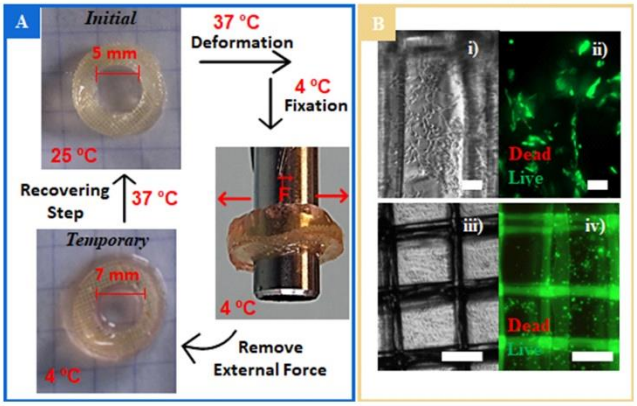

Figure 5. CHTMA-SMPU composite (A) Digital images of ring shape deformation, fixation and recovering steps. (B) Biocompatibility in MC3T3-E1 pre-osteoblasts. i) and ii) Optical and fluorescent images, respectively, of cells seeded on the surface; and iii) and iv) optical and fluorescent images of encapsulated cells after 1 day in culture. In the fluorescence images, live cells were stained with calcein-AM (green) and dead cells with propidium iodide (red). Scale bars: i) and ii) 100 μm; iii) and iv) 200 μm.

ASSOCIATED CONTENT

Supporting Information. Materials, experimental procedures for polymers synthesis, all ^{1}H NMR spectra, polymers-SMPU composites fabrication, shape memory studies and biological assays. This material is available free of charge via the Internet at http://pubs.acs.org.

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The manuscript was written through the contributions of all authors.

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ABBREVIATIONS

CHT, chitosan; CHTMA, methacrylated chitosan; CHTMA-SMPU, methacrylated chitosan-shape memory polyurethane; GEL, gelatin; GELMA, methacrylated gelatin; GELMA-SMPU, methacrylated gelatin-shape memory polyurethane; HA, hyaluronic acid; HAMMA, methacrylated hyaluronic acid; HAMA-SMPU, methacrylated hyaluronic acid-shape memory polyurethane; LAM, laminarin; LAMMA, methacrylated laminarin; LAMMA-SMPU, methacrylated laminarin-shape memory polyurethane; NMR, nuclear magnetic resonance; R_f, shape fixity ratio; Rr, shape recovery ratio; SMP, shape memory polymers; SMPU, shape memory polyurethane; TERM, tissue engineering and regenerative medicine; T_{trans}, transition temperature; UV-Vis, ultraviolet-visible; α-MEM, minimum essential medium α-modification.

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