A nanoconcrete welding strategy for constructing high-performance wound dressing

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\textbf{A B S T R A C T}

Engineering biomaterials to meet specific biomedical applications raises high requirements of mechanical performances, and simultaneous strengthening and toughening of polymer are frequently necessary but very challenging in many cases. In this work, we propose a new concept of nanoconcrete welding polymer chains, where mesoporous CaCO\textsubscript{3} (mCaCO\textsubscript{3}) nanoconcretes which are composed of amorphous and nanocrystalline phases are developed to powerfully weld polymer chains through siphoning-induced occlusion, hydration-driven crystallization and dehydration-driven compression of nanoconcretes. The mCaCO\textsubscript{3} nanoconcrete welding technology is verified to be able to remarkably augment strength, toughness and anti-fatigue performances of a model polymer poly(3-hydroxybutyrate-co-3-hydroxyvalerate)-based porous membrane. Mechanistically, we have revealed polymer-occluded nanocrystal structure and welding-derived microstress which is much stronger than interfacial Van der Waals force, thus efficiently preventing the generation of microcracks and repairing initial microcracks by microcracks-induced hydration, crystallization and polymer welding of mCaCO\textsubscript{3} nanoconcretes. Constructed porous membrane is used as wound dressing, exhibiting a special nanoplates-constructed surface topography as well as a porous structure with plentiful oriented, aligned and opened pore channels, improved hydrophilicity, water vapor permeability, anti-bacterial and cell adherence, in support of wound healing and skin structural/functional repairing. The proposed nanoconcrete-welding-polymer strategy breaks a new pathway for improving the mechanical performances of polymers.

\section{1. Introduction}

Mechanical and biological performances are two key indicators for engineering biomaterials such as bone-implant biomaterials, wound dressing and artificial skin, which often need relatively high strength, toughness and bioactivity [1,2]. Many promising polymers and bio-ceramics with high biocompatibility have to be strengthened and/or toughened to meet the demands of clinical applications. One of the classic methods for strengthening and toughening is to incorporate second-phase nanoparticles in the matrix [3–8]. The interfacial attraction between nanoparticle and polymer chain is the key to block microcrack for toughening. However, the interfacial attractions, including Van der Waals forces, hydrogen bonding and electrostatic pull, are relatively weak and generally lower than the attraction between polymer crystal grains, leading to the limited toughening outcomes and even strength loss. It is a great challenge for simultaneous strengthening and toughening in many cases. In addition, the favorable composition and surface topography of polymer-based composites can endow them with a specific anti-bacterial capability in support of tissue engineering, which can be modulated by incorporating active

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components and inorganic nanomaterials [9–11]. To construct such favorable composition and surface topography is vitally important.

CaCO₃ nanoparticle as a second-phase incorporation material has been well applied in the plastics industry owing to its high availability, low cost and high whiteness, and also exhibits a high potential for biomedical application because of its high biocompatibility and biodegradability [12,13]. However, popular methods of modifying polymers with CaCO₃ nanoparticles still depend on weak interfacial attraction. It is worth noticing that amorphous CaCO₃ exhibits polymer-like flexibility, enabling it to copolymerize with polymers and also to encapsulate micelles, carbon nanodots, gold nanoparticles, macromolecules, small-molecule amino acids and fluoresceins during its crystallization [13–25]. Therefore, we here hypothesize that powerfully welding polymer chains within CaCO₃ nanocrystal through siphoning-induced occlusion, hydration-driven crystallization and dehydration-driven compression of amorphous mesoporous CaCO₃ nanoconcretes will possibly more effectively enhance the strength and toughness of the polymer. However, it is challenging to prepare

Fig. 1. Synthesis and characterization of CaCO₃ nanoconcretes. (a) Schematic illustration of the synthesis of sCaCO₃ and mCaCO₃ nanoconcretes, (b) Scanning electronic microscopy (SEM) image of mCaCO₃ nanoconcretes, (c) Transmission electron microscope (TEM) image of mCaCO₃ nanoconcretes, (d) High-resolution TEM image of mCaCO₃ nanoconcretes, (e) FT-IR spectra, (f) Raman spectra, (g) N₂ adsorption-desorption curves, and (h) pore diameter distributions of sCaCO₃ and mCaCO₃ nanoconcretes.
amorphous mesoporous CaCO\(_3\) nanoparticles as it has not been reported previously.

In this work, we developed an ion-etching method to synthesize a new type of mesoporous CaCO\(_3\) nanoconcretes (mCaCO\(_3\)) composed of amorphous and nanocrystalline CaCO\(_3\) (Fig. 1a), and then constructed a kind of porous membrane by mCaCO\(_3\) welding poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) by a freeze-drying/hydration method (Fig. 2a). The as-constructed porous membrane exhibited remarkably augmented strength, toughness and anti-fatigue performances. Proof-of-concept experimental results verified our hypothesis that the welding of polymer chains with mCaCO\(_3\) made primary contributions to simultaneous strengthening and toughening of polymer. Polymer chains (the welding object) were adsorbed within the porous channels of mesoporous CaCO\(_3\) nanoparticles (mCaCO\(_3\), the welding node) which were composed of amorphous and nanocrystalline phases, and then clamped/welded by the dehydration-driven mechanical force during the crystallization of mCaCO\(_3\), forming a kind of mCaCO\(_3\)-polymer occlusion. The constructed porous membrane as wound dressing also demonstrated a special nanoplates-constructed surface topography in favor of antibacterial and promoted epithelial cell adherence and proliferation, plentiful oriented, aligned and opened pore channels (about 100 nm in diameter) in favor of improved hydrophilicity and water vapor permeability, together in support of wound repairing.

2. Materials and methods

2.1. Chemicals and reagents

Calcium chloride dihydrate (CaCl\(_2\)·2H\(_2\)O), triethyl phosphate (TEP), ammonium bicarbonate (NH\(_4\)HCO\(_3\)) and rhodamine B (RhB) were purchased from Sigma-Aldrich. All other reagents used were of the highest commercial grade available. Cell Counting Kit-8 (CCK-8) and calcein-AM were purchased from Beyotime Biotechnology Co., Ltd. Pure water was produced from a Milli-Q Academic Water Purification System (Millipore Corp., Billerica, MA, USA). Mouse 3T3 fibroblast cells were purchased from Shanghai Zhong Qiao Xin Zhou Biotechnology Co., Ltd.

2.2. Preparation and characterization of mCaCO\(_3\) and sCaCO\(_3\) nanoparticles

A modified gas diffusion method was used to prepare mCaCO\(_3\) and sCaCO\(_3\). At first, CaCl\(_2\)·2H\(_2\)O (5 mM) and TEP (3 mM) were dissolved in 40 mL anhydrate ethanol in a fresh and smooth glass bottle, which was sealed with parafilm with five pinholes. The bottle was put in a container, and then a plentiful amount of NH\(_4\)HCO\(_3\) powder was evenly paved outside the bottle at the bottom of the container, and finally the container was sealed. After static incubation at room temperature for 7 days, the product mCaCO\(_3\) was collected by centrifugation, and washed with anhydrate ethanol for 3 times, and then freshly used for the preparation of porous membrane. sCaCO\(_3\) was synthesized by a similar
method in the absence of TEP.

The morphologies and sizes of mCaCO₃ and sCaCO₃ were observed on scanning electron microscopes (Apero/Nova NanoSEM, FEI) and a transmission electron microscope (Tecnai TF20, FEI). The composition was detected by Fourier transform infrared spectroscopy (Nexus 670, Thermo-Nicolet), Raman spectroscopy (Renishaw inVia Raman Microscope), X-ray diffraction (Ultima IV, Rigaku), and elemental mapping were collected using HT7700 (Hitachi) and Titan Cubed Themis G2 300 (FEI). Contact angles of porous membranes and elementary mapping were collected using a multi-channel synchronic data machine (Shenzhen Henwiton Electronic Technology Co., Ltd.) with a sampling frequency of 120 Hz by a confocal laser scanning microscope (ZEISS LSM880) and then reconstructed.

2.3. Preparation and characterization of porous membranes

Porous membranes were prepared by a freeze-drying method. PHBV (75 mg) was fully dissolved in dichloromethane (DCM, 3 mL), and CaCO₃ nanoparticles (15 mg) were thoroughly dispersed into the above solution. The mixture solution was poured into a home-made smooth stainless steel mold (7 cm × 3 cm × 3 cm) which was wrapped with tin foil and then put into a heat preservation box to keep on the horizon. A plentiful amount of liquid nitrogen was quickly poured into the box to freeze the polymer solution. The mold-contained box was rapidly transferred to a constant temperature vacuum drying oven to vacuumize the membrane for 30 min and then maintain vacuum overnight in order to completely remove DCM. Finally, the porous membrane was pulled off from the mold carefully, and then immersed into deionized water for 2 days. Water on the surface of the porous membrane was drained dry and then as-prepared porous membranes were stored for use. A bigger dimension of mold (20 cm × 20 cm × 5 cm) was also made to prepare a big area of the porous membrane without quality loss.

The surface microstructure patterns of porous membranes were observed on a scanning electron microscope (Apero, FEI). As to microstructure measurement, porous membranes were embedded into resin, and then cut into 60 nm-thick membranes to put on glass sheets or TEM copper meshes for various measurements. High-resolution TEM images and elementary mapping were collected using HIT7700 (Hitachi) and Titan Cubed Themis G2 300 (FEI). Contact angles of porous membranes were measured on a Dynamic Contact Angle Meter (XG-CAMC, Shanghai Xuanzhu Instrument Co., Ltd.).

2.4. 3D fluorescence imaging of pore structure of porous membrane

Dry porous membrane was immersed in an ethanolic solution of RhB to be vacuumized for 2 h. Residual RhB on the surface of the porous membrane was washed away with ethanol. After oil seal on a glass sheet, a three-dimensional structure of the porous membrane was scanned layer by layer on a two-photon confocal laser scanning microscope (ZEISS LSM880) and then reconstructed.

2.5. Measurement of mechanical performances

The porous membrane was cut into a strip with a standard dimension of 15 cm × 5 cm, and then fixed with a clamp at an equal distance on both ends. Tensile measurement was executed at a stretch speed of 5 mm/min on a UTM2012 high-resolution universal tensile testing machine (Shenzhen Henwitron Electronic Technology Co., Ltd.) with a sampling frequency of 120 Hz by a multi-channel synchronous data collection mode, and stopped until porous membrane was completely broken. As for anti-fatigue bending measurement, the porous membrane was bent 3 mm in the vertical distance and then unfolded for location recovery at a speed of 30 mm/min for 500 cycles on the universal tensile testing machine. At the maximal bending strength, a circularly bent porous membrane was used to measure its maximal tensile strength.

2.6. Measurement of the permeability of porous membranes

The water vapor permeability of porous membranes was measured by the differential-pressure method. A round-mouth glass bottle was filled with water and then sealed with porous membranes to achieve an effective transmission area of 1.54 cm². The bottle was placed in a vacuum drying oven to maintain at 1 atm at 25 ℃ for 5 h, and then weighted water loss to calculate the water vapor transmission.

2.7. Antibacterial performance measurement

Escherichia coli and Staphylococcus aureus were cultured to log phase (10⁶ CFU/mL) and diluted 1,000 times to prepare bacterial suspensions in LB liquid medium. Porous membranes were cut into discs with 5 mm in diameter by a hole punch and put in a 96-well plate (n = 6). A diluted bacterial suspension was added into wells (100 μL/well), and then incubated at 37 ℃ for 24 h. The absorbance at 600 nm was measured on a microplate reader (Synergy H1M, Bio-Tek) to determine bacterial concentration.

2.8. Measurement of cytotoxicity and cell proliferation

As to cytotoxicity measurement, a 5 mm-diameter circular disk of the porous membrane (n = 6) was put in the well of 96-well plates after sterilization, and then 10⁴ 3T3 cells or MCF-10A cells were added into each well. After incubation at 37 ℃ under an atmosphere of 5% CO₂ and 90% relative humidity in an incubator (Thermo Scientific) for 24 h, CCK-8 reagent was used to detect the absorbance at 450 nm using the Bio-Tek microplate reader and then to calculate cytotoxicity by subtracting the background prior to the addition of CCK-8.

As for cell adherence and proliferation measurement, a 1.5 cm-diameter circular disk of the porous membrane was placed in a 12-well plate after sterilization, and then 4 × 10⁵ 3T3 cells were added into each well. Culture medium was replaced with a fresh one once 48 h. After continuous culture for 7 days, porous membranes were taken out, and un-adhered cells were washed away with PBS gently and then stained with calcein-AM for fluorescence observation (488 nm/515 nm as excitation/emission wavelengths) on a confocal laser scanning microscope (TCS SP5 II).

2.9. Animal experiments

About 4-week old female BALB/c mice (~20g, purchased from Guangdong Medical Laboratory Animal Center) were randomly divided into four groups (n = 12), Control, PHBV, sCaCO₃@PHBV, and mCaCO₃-PHBV. After anesthesia by intraperitoneal injection 100 μL of 10% chloral hydrate and subsequent removal of the hair on the back, a 1 cm-diameter wound was created using a special hole punch to remove the whole cortex on a sterile operation platform. After being stuck with a porous membrane as wound dressing, the wound was wrapped with gauze and bandage, and then observed at Day 1, 5, 7, 10, and 13, respectively. After treatment for 13 days, mice were humanely sacrificed, and then the skin on healed wound was removed and fixed with 4% paraformaldehyde, and then the slice of the healed skin at the center of the wound was stained by H&E and Masson methods for histological analysis. In addition, several main organs (heart, liver, spleen, lung and kidney) were collected, fixed with 4% paraformaldehyde, and stained with H&E for histological analysis. Moreover, Before humane execution after 13 day treatment, the blood of each mouse was collected and then used for assessment of liver/kidney functions and hemotoxicity on a biochemical analyzer (iMagic-M7) and a blood cell analyzer (BC-31S, Mindray).

3. Results and conclusion

3.1. Synthesis and characterization of CaCO₃ nanoconcretes

A classical gas diffusion method was used to synthesize CaCO₃ nanoconcretes using CaCl₂·2H₂O as calcium/water sources and NH₄HCO₃ as carbonate/water sources (Fig. 1a). In order to obtain stable
amorphous CaCO₃ nanoparticles, ethanol was used as the solvent instead of water because it is hard for them to stabilize in an aqueous solution. Moreover, ethanol has a relatively lower dielectric constant (24.5) than water (78.5), and can therefore confine the dissociation/ionization of all reactants, reducing reaction rate in favor of controllable uniform growth of CaCO₃ nanoparticles. In this system, additive water except CaCl₂·2H₂O and NH₄HCO₃ will easily lead to unwanted CaCO₃ crystallization. Ethanol, H₂O, Cl⁻ and NH₄⁺ all can coordinate with Ca²⁺ and consequently inhibit CaCO₃ precipitation together in support of controlled growth with about 3 days of the incubation period for visible precipitation [26]. Unexpectedly, we found from IR (Fig. 1e), Raman (Fig. 1f), XRD (Supplementary Fig. S1) and TEM (Supplementary Fig. S2) measurements that the synthesized CaCO₃ nanoparticles were a mixture of amorphous calcium carbonate (ACC), aragonite and calcite nanocrystals, which was rarely observed previously, although similar CaCO₃ nanoparticles were synthesized under similar conditions by the same method by many other researchers [27–31]. In addition, collected solid CaCO₃ (sCaCO₃) nanocomposites can be recrystallized to submicron calcite crystals by the hydration and dehydration evolutions when transferred to an aqueous solution (Supplementary Fig. S3). Such a mixture structure and a consolidation behavior are quite similar to that of concrete, and therefore we called them nanoconcretes in this work.

Furthermore, we attempted to add etchants to obtain a porous structure of CaCO₃ nanoconcretes. Triethyl phosphate (TEP) was selected as an etching agent because its hydrolysis products phosphates are a poison able to effectively inhibit CaCO₃ precipitation by highly competing with carbonate ions for coordination with Ca²⁺ (Fig. 1a) as calcium carbonate normally does not precipitate from phosphates-rich seawater with 300% supersaturation [32–34]. From SEM and TEM images (Fig. 1bc and Supplementary Fig. S4), we can clearly find that CaCO₃ nanoconcretes which were synthesized in the presence of TEP had high porosity (mesoporous CaCO₃ nanoconcretes, mCaCO₃). Moreover, from Brunauer-Emmett-Teller (BET) measurement results (Fig. 1gh), mCaCO₃ exhibited an obvious mesoporous structure with porous size of 2–20 nm (about 10 nm in average pore size) and higher specific surface area (417 cm²/g) compared with sCaCO₃ (81 cm²/g) in accordance with the above SEM observation results. From TG results (Supplementary Fig. S5), we further found that the water content of mesoporous CaCO₃ nanoconcretes (mCaCO₃) was lower than that of the above-mentioned solid CaCO₃ nanoconcretes (sCaCO₃), indicating that mCaCO₃ had a higher proportion of ACC, owing to stronger inhibition effect of phosphates against aragonite and calcite crystals. The environment-sensitive buffered system can induce the dissolution-reprecipitation evolution during gas diffusion [35–37], which was possibly the main reason that phosphates can drill pores in the ACC phase. A dilute ethanol solution of water (volume ratio of water to ethanol = 5:95) was used to check the phase transformation behavior of mCaCO₃, which experienced a phase transformation process involving ACC hydration/dissolution and then dehydration/crystallization, aragonite fusion/growth, and calcite formation (Supplementary Fig. S6). Such a concrete-like quick consolidation behavior and a porous structure will ensure mCaCO₃ to weld polymer chains occluded within the mesoporous channels.

3.2. Synthesis and microstructure characterisation of mCaCO₃-PHBV membrane

Next, PHBV was chosen as a model polymer to construct wound dressing with mCaCO₃ nanoconcretes together because PHBV has excellent biodegradation and high biocompatibility but relatively low toughness [38]. A freeze-drying method was developed to quickly crystallize the solvent dichloromethane (DCM) and PHBV under cooling of liquid nitrogen, and then to form the mCaCO₃-encapsulated PHBV membrane with porous structure after quick sublimation/removal of DCM crystals under vacuum (Fig. 2b) [39,40]. Then the mCaCO₃-encapsulated PHBV membrane was immersed into water to locally crystallize mCaCO₃ nanoconcretes into calcite nanocrystals in a confined space, which occluded and welded the encapsulated chains of PHBV to form the mCaCO₃-welding PHBV membrane (mCaCO₃-PHBV) (Fig. 2b). As a control, the sCaCO₃-encapsulated PHBV membrane (sCaCO₃@PHBV) was also prepared by the same procedure. From TEM images of 60 nm-thick transverse section, both mCaCO₃-PHBV and sCaCO₃@PHBV exhibited high porosity with about 100 nm in diameter and high dispersion of calcite nanocrystals (Fig. 2b and Supplementary Fig. S7). Three-dimensional two-photon fluorescence imaging further discovered that pore channels were plentiful, oriented, aligned and opened (Fig. 2c and Supplementary Movie S1), which can be due to oriented and uniform crystallization of DCM under gradient cooling. Such a porous structure should be greatly favorable to improve hydrophilicity and water vapor permeability of PHBV membrane in imitation of partial skin functions. From high-resolution TEM images (Fig. 2de) and elementary mapping (Fig. 2f) of 60 nm-thick section of mCaCO₃-PHBV, PHBV chains were indeed incorporated into the crystal lattice of calcite nanocrystals as indicated by internal crystal deficiencies (yellow circles in Fig. 2de) and extra oxygen signal (Fig. 2f) outside of Ca (basically reflects oxygen in CaCO₃) within crystal where red without overlap with green belongs to occluded PHBV. By comparison, PHBV chains in the sCaCO₃@PHBV membrane only surrounded calcite nano-crystals rather than incorporated into their crystal lattice ( Supplementary Fig. S8).

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3.3. Mechanical performances and welding mechanisms of mCaCO₃-PHBV membrane

Furthermore, mechanical performances of membranes were evaluated from microscopic to macroscopic levels. At first, to detect the interfacial attractions between calcite nanocrystals and PHBV chains, the strain mappings across single CaCO₃ crystals in the 60 nm-thick slices of porous membranes were determined by geometric phase analysis (GPA). The percentage degrees of in-plane fringe deformation (εₓₓ), out-of-plane fringe deformation (εᵧᵧ), fringe rotation (Rᵧᵧ), and shear strain (Sᵧᵧ) were evaluated with respect to a reference area (εₓₓ = 0%, εᵧᵧ = 0%, Rᵧᵧ = 0°, and Sᵧᵧ = 0%) arbitrarily selected in the nanocrystal center, and used to construct corresponding topological maps of strain distribution. In these maps, the color scale and the signs “+” and “−” denote, respectively, the extent and the direction of a particular strain component change, relative to the reference. From Fig. 3a, the εₓₓ, εᵧᵧ, Rᵧᵧ and Sᵧᵧ components in the sCaCO₃@PHBV sample generally exhibited weak oscillations which were discretely distributed in the nanocrystal, possibly owing to the formation of lattice fringe distortion during the concrete-like quick consolidation. By comparison, these components in the mCaCO₃-PHBV membrane sample displayed relatively stronger strains. Noticeably, the concerned fringes spanned the entire nanocrystal section in a continuous way, which should be attributed to the incorporation of polymer chains into calcite nanocrystal in accordance with the high-resolution TEM results in Fig. 2d–f. Therefore, it can be concluded that a strong strain of nanocrystal welding polymer chains was formed during the concrete-like quick consolidation. Such a microscopic nanocrystal-welding-polymer force would make a contribution to the macroscopic mechanical performances of mCaCO₃-PHBV membrane.

From Supplementary Fig. S9a, compared with the common natural drying method, the freeze-drying method can distinctly enhance the tensile strength and elongation of PHBV membrane, which can be attributed to quick freeze-drying-induced reduction in PHBV grain size as XRD peak width at half height decreased (Supplementary Fig. S10). The incorporation of CaCO₃ nanoparticles further decreased PHBV grain size (Supplementary Fig. S10) and thus freshly prepared sCaCO₃@PHBV membrane can mildly improve the tensile strength of PHBV membrane (1.3 fold, Supplementary Fig. S9). Besides PHBV crystallization,
hydration-driven mCaCO$_3$ crystallization and PHBV welding made more remarkable contributions to both strengthening (2.1 fold) and toughening (Fig. 3c, Supplementary Fig. S9b–d) in accordance with GPA results. 

Anti-fatigue performances were further evaluated by 500-cycle continuous bending (Supplementary Movie S2) since they are very important to tissue engineering, especially wound dressing. From Supplementary Fig. S11 and Fig. 4a, brittle fracture occurred in PHBV membrane after 99 cycles of bending, while it occurred in sCaCO$_3$@PHBV membrane after 352 cycles of bending. Strikingly, no brittle fracture was observed in mCaCO$_3$-PHBV membrane after bending strength began to attenuate at 268 cycles. To track the change in mechanical performances during bending, we collected data at three points, the initial point before bending (Fig. 3c), the maximal/critical point where bending strength arrived at the maximum (Fig. 3d), and the final point after 500 cycles of bending (Fig. 3e). It can be found that both tensile strength and elastic elongation of PHBV gradually decreased during the bending cycle, but that of sCaCO$_3$@PHBV membrane and mCaCO$_3$-PHBV membrane firstly increased and then decreased, and always followed the law of mCaCO$_3$-PHBV > sCaCO$_3$@PHBV > PHBV (Fig. 3g,h,i). Noticeably, elongation after yielding of mCaCO$_3$-PHBV membrane always increased during bending cycle (Fig. 3h) and became
remarkably more than that of sCaCO$_3$@PHBV membrane after 500 cycles (orange shadow in Fig. 3g), indicating that bending mCaCO$_3$-PHBV membrane gradually enhanced its toughness and meanwhile maintained a relatively high strength, owing to powerful welding force. Such simultaneous strengthening, toughening and anti-fatigue effects are quite significant to realize high mechanical performances of wound dressing.

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Moreover, in order to make clear the mechanism of strengthening, toughening and anti-fatigue effects, we further tracked and analyzed mechanical behaviors of membranes during bending cycles. From tendency charts in Fig. 4b extracted from original data in Fig. 4a, distinctly different from brittle fracture behavior of PHBV, mCaCO$_3$-PHBV and sCaCO$_3$@PHBV membranes experienced the cycles of transient generation and repair of microcracks, where there were three steps in each cycle: energy accumulation (I), energy release from microcracks (II), and energy absorption by nanoparticles (III).

Fig. 4. Anti-fatigue performances and mechanisms of porous membranes. (a) Bending strength change of porous membranes during 500-cycle continuous bending. (b) Tendency charts extracted from Fig. 4a, where three steps included in each strength change cycle represent energy accumulation (I), energy release from microcracks (II), and energy absorption by nanoparticles (III). (c) STEM images of 60 nm-thick slices of mCaCO$_3$-PHBV membranes before bending and after bending for 268 cycles. Scale bar, 2 μm. Yellow circles and blue arrows represent non-crystallization and crystallization of mCaCO$_3$ nanoconcretes, respectively. (d) Schematic diagram of anti-fatigue mechanisms of mCaCO$_3$-PHBV membrane.
cycle (purple arrows in Fig. 4b): Step I, energy accumulation; Step II, energy release from microcracks; Step III, energy absorption by nanoparticles. It is obvious that both sCaCO₃ and mCaCO₃ nanocrystals as a second phase effectively blocked the expansion of microcracks by absorbing their energy, increasing the toughness of PHBV membrane. Compared with sCaCO₃@PHBV membrane, mCaCO₃-PHBV membrane can more quickly (reflected by more cycle times, Fig. 4b) absorb higher energy (reflected by the area under the curve, Fig. 4b), more efficiently enhancing the toughness. It should originate from a stronger interfacial binding force between mCaCO₃ nanocrystal and PHBV chains. In addition, after elastic yield, mCaCO₃-PHBV membrane exhibited higher plasticity than sCaCO₃@PHBV membrane, as suggested by bigger area of shadow under the curve in Fig. 4b (orange versus blue), and also maintained remarkably higher strengths than sCaCO₃@PHBV membrane.

Notably, elongation after yielding of mCaCO₃-PHBV membrane always increased during bending cycle (Fig. 3g) and became remarkably more than that of sCaCO₃@PHBV membrane after 500 cycles (orange shadow in Fig. 3g), indicating that bending mCaCO₃-PHBV membrane gradually enhanced its toughness and meanwhile maintained a relatively high strength, owing to powerful welding force. Such simultaneous strengthening, toughening and anti-fatigue effects are quite significant to realize high mechanical performances of wound dressing. Moreover, before yielding (Fig. 4b), the difference of strength between mCaCO₃-PHBV and sCaCO₃@PHBV membrane overall became bigger and bigger with the increase of energy-absorption cycle (Supplementary Fig. S12), suggesting that there was another factor besides bending-induced polymer hardening making a contribution to the strengthening of mCaCO₃-PHBV membrane during bending. Therefore, we further checked the microstructure of mCaCO₃-PHBV membrane at the

![Fig. 5. Biological performances of porous membranes.](image)

(a) Surface topography (Scale bar, 5 μm), (b) Hydrophilicity, (c) Water vapor permeability, (d) Cytotoxicity to 3T3 cells, (e) fluorescence images of 3T3 cell adherence and proliferation on PHBV, sCaCO₃@PHBV and mCaCO₃-PHBV membranes (Scale bar, 100 μm), and (f) Corresponding quantitative analysis. P values were calculated by the two-tailed Student’s t-test (*p < 0.05, **p < 0.01, ***p < 0.0001; NS, no significant difference).
critical yield point. We found that there were partial mCaCO₃ nanoconcretes away from PHBV pores not crystallized yet (as indicated by yellow circles in Fig. 4c), but they became crystalline after 268-cycle bending (as indicated by blue arrows in Fig. 4c). We think that it resulted from microcracks-induced penetration of water from PHBV pores to mCaCO₃ nanoconcretes and subsequent hydration-induced mCaCO₃ crystallization and welding with PHBV (Fig. 4d). The case of sCaCO₃@PHBV membrane was similar (Supplementary Fig. S13). In this period, the energy of microcracks can not only be absorbed by mCaCO₃ nanoconcretes, but also induce their crystallization/welding to repair microcracks for strengthening. Such a bending-driven strengthening and toughening effect is quite interesting and unexpected in great favor of the clinical application of wound dressing.

3.4. Biological performances of mCaCO₃-PHBV membrane as wound dressing

Biological performances of mCaCO₃-PHBV membrane, including hydrophilicity, gas transmission, cell adherence and anti-bacterial were explored. Surface microstructure/topography is a very important factor influencing these performances. From Fig. 5a, PHBV, sCaCO₃@PHBV and mCaCO₃-PHBV membranes exhibited a kind of rough and porous surface with nanoplates-constructed microstructure, and the surface roughness of PHBV membrane was higher than that of sCaCO₃@PHBV and mCaCO₃-PHBV membranes owing to bigger PHBV grain size (Supplementary Fig. S10b). Nanoplate morphology suggested oriented growth of PHBV crystals in accordance with XRD results (Supplementary Fig. S10a). Different from general PHBV materials with high hydrophobicity (contact angle of about 129°) [41], PHBV membrane in this work exhibited a remarkably lower contact angle of 79° (Fig. 5b), possibly owing to the porous structure and rough nano-/micro-structure. Compared PHBV membrane, sCaCO₃@PHBV and mCaCO₃-PHBV membranes displayed a lower contact angle (Fig. 5b), which could be attributed to CaCO₃ incorporation, demonstrating higher hydrophilicity in support of water vapor transmission (Fig. 5c). Moreover, PHBV, sCaCO₃@PHBV and mCaCO₃-PHBV membranes had no visible cytotoxicity to both 3T3 fibroblast cells (Fig. 5d) and MCF-10A endothelial cells (Supplementary Fig. S14). mCaCO₃-PHBV membrane can best induce the adherence (Supplementary Fig. S15) and proliferation (Fig. 5e) of 3T3 fibroblast cells compared with PHBV and sCaCO₃@PHBV membrane, owing to its highest hydrophilicity and fine surface nano-/micro-structure [42]. The order stripes of the membrane in the bright field images in Fig. 5e, which were derived from the surface structure of model, were on the opposite side of cellular adhesion, and

Fig. 6. In vivo wound therapy effect of porous membranes as wound dressing. (a) Photographs of wounds treated for 1, 5, 7, 10 and 13 days, (b) wound area during treatment, and (c) vertical section of healed skin at the center of wound stained by H&E and Masson methods (scale bar, 500 μm). P values were calculated by the two-tailed Student’s t-test (*p < 0.05; NS, no significant difference).
therefore could not cause the orientation growth of cells as indicated by the fluorescence images in Fig. 5e. In addition, PHBV had a moderate anti-bacterial ability \[ 43 \], and mCaCO\(_3\)-PHBV membrane exhibited a better anti-bacterial effect against Escherichia coli and Staphylococcus aureus (Supplementary Fig. S16), which possibly resulted from its specific nanoplate morphology \[ 44,45 \].

Moreover, wound-healing performances of mCaCO\(_3\)-PHBV membrane as wound dressing were investigated using a wound model of mice. From Fig. 6a and b, mCaCO\(_3\)-PHBV membrane can accelerate wound healing faster compared with blank control. The mCaCO\(_3\)-PHBV membrane maintained higher integrity during the whole therapy period compared with PHBV and sCaCO\(_3@PHBV\) membranes (Fig. 6a), owing to its higher mechanical performances, in favor of more effectively blocking the invasion of bacteria and inducing the adherence and growth of cells. Although wounds in all treatment groups healed up finally, histopathologic examination of sections in the middle of wounds indicated that blank control caused severe fibrosis with rarely visible skin appendage, PHBV group still exhibited partial fibrosis, sCaCO\(_3@PHBV\) group, repaired wound to a certain extent without obvious skin appendage, while mCaCO\(_3\)-PHBV membrane induced the full growth of epidermis, hypoderim and skin appendage with plentiful hair follicles, glandular integumentaria and clear blood vessels (Fig. 6c). Therefore, mCaCO\(_3\)-PHBV membrane can effectively improve wound healing by inducing the integrated adherence and substantial growth of functional cells because of high hydrophilicity and roughness rather than by causing fibrosis, and thus mCaCO\(_3\)-PHBV-repairing wound will have better biological functions of the skin. In addition, mCaCO\(_3\)-PHBV membrane exhibited high blood compatibility and invisible toxicity to main normal organs (Supplementary Fig. S17–19).

4. Conclusion

In summary, we developed an ion etching method to synthesize a kind of mesoporous CaCO\(_3\) nanoconcretes composed of amorphous and nanocrystalline phases, and utilized them to construct a type of porous organic/inorganic membrane by a freeze-drying/hydration method. The polymer welding effect of mesoporous CaCO\(_3\) nanoconcretes was confirmed to play a primary role in strengthening and toughening the porous PHBV membrane. Mechanical and biological performances were improved by the welding strategy, and the constructed mCaCO\(_3\)-PHBV membrane was used as wound dressing, exhibiting ideal wound repair outcomes. The proposed nanoconcrete-welding-polymer concept delivers a promising strategy for improving the mechanical performances of polymers. In principle, this is a general platform technology which is possibly extendable to be applied in various macromolecular matrices.

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Appendix A. Supplementary data

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