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Low molecular weight hydrogels derived from urea based-bolaamphiphiles as new injectable biomaterials

Michael A. Ramin [a, b], Laurent Latxague [a, b], Kotagudda Ranganath Sindhu [c], Olivier Chassande [c], Philippe Barthélémy [a, b, *]

[a] Univ. Bordeaux, ARNA Laboratory, F-33016 Bordeaux, France
[b] INSERM U1212, CNRS UMR 5320, ARNA Laboratory, F-33016 Bordeaux, France
[c] INSERM, U1226, Biotis Laboratory, F-33016 Bordeaux, France

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ABSTRACT

There is a critical need for soft materials in the field of regenerative medicine and tissue engineering. However, designing injectable hydrogel scaffolds encompassing both adequate mechanical and biological properties remains a key challenge for *in vivo* applications. Here we use a bottom-up approach for synthesizing supramolecular gels to generate novel biomaterial candidates. We evaluated the low molecular weight gels candidates *in vivo* and identified one urea-containing molecule, compound **16**, that avoid foreign body reactions in mice. The self-assembly of bolaamphiphiles creates a unique hydrogel supramolecular structures featuring fast gelation kinetics, high elastic moduli, thixotropic, and thermal reversibility properties. This soft material, which inhibits recognition by macrophages and fibrous deposition, exhibits long-term stability after *in vivo* injection.

1. Introduction

The development of artificial materials for repairing, replacing or regenerating damaged tissues remains an important issue in the field of regenerative medicine. These materials mimic natural extracellular matrix (ECM) [1], which can be seen, as a soft matter constituted of extracellular molecules such as proteins or polysaccharides secreted by cells. These biomolecules form a macromolecular network providing a support to the surrounding cells. Likewise, engineered hydrogels that resemble to the physicochemical characteristics of ECM have been used as scaffold in the field regenerative medicine [2]. Many different natural polymers have been used to develop hydrogels, including collagen [3], alginate [4,5] hyaluronic acid [6], chondroitin sulfate [7], and fibrin [8], for example. Also synthetic polymers and/or copolymers have been used to fabricate scaffolds. In this category, one can cite poly(lactic acid) [9,10], poly(ethylene glycol) [11] and poly(glycolic acid) [12,13]. In order to improve the biological, biophysical and mechanical properties of these materials, chemically modified natural scaffolds [14] or hybrid hydrogels [15,16] combining natural and synthetic hydrogels have also been investigated.

Recently, a polymer free approach involving Low Molecular Weight Gels (LMWGs) has attracted great interests as novel scaffolds for biomedical applications [17–22]. These biocompatible materials, which result from the self-assemblies of small molecules possess tunable physicochemical and biological properties thanks to their supramolecular structures. Despite the first promising results reported with LMWGs [23–27], implementing injectable hydrogel scaffolds able to i) encompass the requested biophysical and mechanical properties and ii) avoid foreign body reactions remains highly desirable.

The purpose of this study was to investigate the use of novel bolaamphiphile based LMWGs possessing amide and urea functions as injectable biomaterials for *in vivo* applications. Herein, we report the synthesis of novel bolaamphiphiles featuring N-thymine glycosylated head groups linked to a lipidic moiety *via* either urea or amide functions (Fig. 1). These chemical functions impact on both mechanical and biological properties of their resulting supramolecular hydrogels. Contrary to previous bolaamphiphiles [22,28,29], the urea-containing molecules feature a fast gelation kinetic and high *in vivo* stability. The urea-based-bolaamphiphile

* Corresponding author. Univ. Bordeaux, ARNA Laboratory, F-33016 Bordeaux, France.
E-mail address: philippe.barthelemy@inserm.fr (P. Barthélémy).

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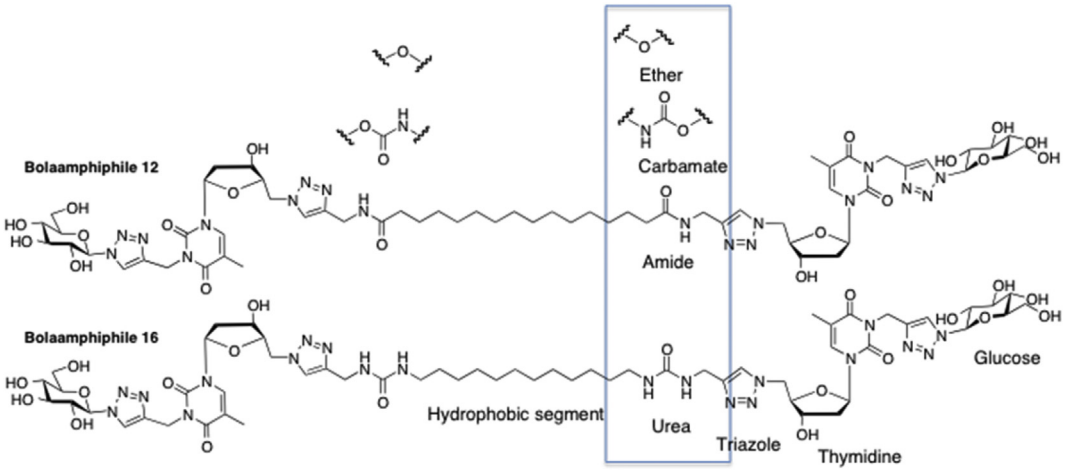

Fig. 1. Chemical structures of bolaamphiphiles. Examples of structures amide and urea based Bolaamphiphiles (**12** and **16**) reported in this study. Ether and carbamate analogues were reported previously (see ref 22 and 28, respectively).

forms a hydrogel that inhibits recognition by macrophages and fibrous deposition.

2. Experimental section

2.1. Materials and measurements

All commercially reagents and solvents (Fluka, Sigma-Aldrich, Alfa-Aesar) were used without further purification. For reactions requiring anhydrous conditions, dry solvents were used under inert atmosphere (nitrogen or argon). Analytical thin layer chromatography (TLC) was performed on pre-coated silica gel F254 plates with fluorescent indicator (Merck). The detection of compounds was accomplished with UV light (254 nm). All compounds were characterized using ^{1}H and ^{13}C Nuclear Magnetic Resonance (NMR) spectroscopy (Bruker Avance DPX-300 spectrometer, 1H at 300.13 MHz and ^{13}C at 75.46 MHz). Assignments were made by ^{1}H-^{1}H COSY (*correlation spectroscopy*), DEPT (*distortionless enhancement by polarization transfer*) and HSQC (*heteronuclear single quantum correlation*) experiments. Chemical shifts (δ) are given in parts per million (ppm) and all the spectra were referenced to the residual proton signals and referenced to residual solvent peaks (δ_H = 7.26 ppm and δ_C = 77.0 ppm for $CDCl_3$). Coupling constants J are given in Hertz (Hz). The multiplicity of each signal is designated using the following abbreviations: s = singlet; d = doublet; t = triplet; q = quartet; m = multiplet. High-resolution mass spectra (HRMS) were recorded with a Q-Exactive mass spectrometer (Thermo Fisher Scientific) in the electrospray ionisation (ESI) mode at the Center Régional de Mesures Physiques de l'Ouest (CRMPO, Université de Rennes).

2.2. Synthesis

The general procedure for synthesis of bolaamphiphiles is shown in Scheme 1, the detailed procedures are described in Supporting Information.

2.3. Physico-chemistry and spectroscopy studies

2.3.1. Critical gelation concentration (CGC) and gelation kinetic

The gelation ability was quantitatively assessed by investigating the critical gelation concentration (CGC), which is defined as the lowest concentration (in weight percent) of hydrogelator required to gelify 1 mL of water. For the CGC measurement, the hydrogels were successively diluted, heated until no formation of gel. The sample is considered to be a gel by the tube-inverting method.

Kinetics. All samples (1% w/w) were first heated above their sol-gel transition temperature (80 °C, 5 min) and then allowed to cool to room temperature (23 °C). The gelation kinetic corresponds to the time necessary for the gel formation.

2.3.2. Differential scanning calorimetry (DSC)

DSC was performed using a Mettler Toledo analyzer (DSC 1 STARe System, Singapore). Samples were generally sealed inside aluminium crucibles of 100 μL in volume and heated from 20 to 85 °C at a heating rate of 1 °C/min. Second heating run was selected for analysis.

2.3.3. IR spectroscopy

A Perkin-Elmer Spectrum Two FT-IR spectrometer with an attenuated total reflection (ATR) accessory was used to record the IR spectra. To perform infrared experiments on hydrogels, H_2O was replaced by D_2O to overcome the strong water absorption (at 1640 cm^{-1}), which can affect the analysis.

2.4. Mechanical property measurement of hydrogels

Rheological measurements were carried out on a Malvern Kinexus Pro+ rheometer with steel plate-plate geometry (diameter: 20 mm). The lower plate is equipped with a Peltier temperature control system, and all samples were studied at 25 ± 0.01 °C unless otherwise indicated. All measurements were conducted with a 0.3 mm gap distance between the plates. A solvent trap was also used to ensure homogeneous temperature and to prevent water evaporation. The hydrogels were heated at 85 °C and the resulting liquid was immediately placed into the rheometer and subjected to sinusoidal oscillations. All experiments were carried out within the linear viscoelastic regime (LVR), in which the measured shear moduli (G′, G″) are independent of the applied strain (i.e. without disruption of the gel structure). For this purpose an amplitude strain sweep (from 0.01 to 100% at an angular frequency of 6.28 rad s^{-1}) was performed for each sample.

2.5. Transmission electronic microscopy (TEM)

TEM microscopy experiments were performed with a Hitachi H