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New hyaluronic acid based brush copolymers synthesized by atom transfer radical polymerization

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ABSTRACT

In this work, an efficient method for the synthesis of hyaluronic acid based brush copolymers using atom transfer radical polymerization (ATRP) has been reported. At first, two different hyaluronic acid (HA) based macroinitiators have been prepared and then they have been used for the polymerization via ATRP of hydrophilic or hydrophobic molecules carrying vinyl portions.

In particular, by linking 2-bromo-2-methylpropionic acid (BMP) to the primary hydroxyl groups of tetrabutyl ammonium salt of HA (HA–TBA) or to amino groups of the ethylenediamino derivative of HA–TBA (HA–TBA–EDA), two macroinitiators (HA–TBA–BMP and HA–TBA–EDA–BMP) have been obtained. Then they have been used for the ATRP of poly(ethylene glycol) methacrylate (PEGMA), butyl methacrylate (BUTMA) or N-isopropylacrylamide (NIPAM) using a complex of Cu(I) and 2,2′-Bipyridyl (Bpy), as a catalyst.

Both macroinitiators and final copolymers, named as HA–BMP–pPEGMA, HA–BMP–pBUTMA, HA–BMP–pNIPAM, HA–EDA–BMP–pPEGMA, HA–EDA–BMP–pBUTMA and HA–EDA–BMP–pNIPAM, have been characterized by spectroscopic analysis and size exclusion chromatography to confirm the success of the polymerization process.

1. Introduction

Hyaluronic acid (HA) is a naturally occurring linear polysaccharide consisting of alternating disaccharide units of α-1,4-D-glucuronic acid and β-1,3-N-acetyl-D-glucosamine (Knudson & Knudson, 2001) that can be found in connective tissues such as umbilical cord, synovial fluid, vitreous, etc. (Laurent, 1970).

As a major component of the extracellular matrix (ECM), HA has been recognized as an important molecule to control cellular differentiation and proliferation as well as the inflammatory response and cellular motility (Fraser,Laurent, & Laurent, 1997;Turley, 1989).

Thanks to its peculiar properties, in the last decades, HA derivatives have been extensively employed for the production of drug delivery systems (Pitarresi et al., 2010; Pitarresi, Craparo, Palumbo, Carlisi, & Giammona, 2007) and scaffolds for tissue engineering (Ji et al., 2006; Palumbo et al., 2012).

However, the pronounced hydrophilic character of HA and its polyanionic nature do not promote cell attachment and subsequent tissue formation (Shu, Liu, Palumbo, & Prestwich, 2003), therefore there is the necessity to modify its chemical structure with molecules able to increase affinity toward cells thus allowing their adhesion and differentiation in the case of tissue engineering. On the other hand, the derivatization of HA with lipophilic molecules can produce amphiphilic derivatives able to self assemble in aqueous medium and to entrap poorly water soluble drugs, thus allowing their administration and modified release in physiological fluids.

These chemical modifications have been mostly performed on HA through grafting techniques and, in despite of several works published in this field by employing “grafting to” technique, the control of molecular weight (MW) and polydispersity index (PDI) is still a major issue that need to be addressed since these two parameters affect the chemical, physical and biological properties of the final product.

Atom Transfer Radical Polymerization (ATRP), discovered by Matyjaszewski and Sawamoto in 1995 (Wang & Matyjaszewski,

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1995), is a versatile controlled radical polymerization (CRP) process. It enables a precise control of MW, PDI and functionality (Coessens, Pintauer, & Matyjaszewski, 2001).

In this technique a transition metal complexed by an appropriate ligand is used as a catalyst for the reaction between an alkyl halide initiator and a vinyl monomer. The reaction can be carried out in a variety of solvents and conditions, including water at room temperature. ATRP has been exploited as homogeneous/heterogeneous solution polymerization technique, as well as "growing from" polymerization technique for molecule in solution, surfaces, proteins, organic and inorganic materials (Cavallaro, Licciardi, Di Stefano, Pitarresi, & Giammona, 2009;Pyun & Matyjaszewski, 2001).

Because ATRP can provide site-specific grafting to a variety of surfaces (essentially any surface containing an ATRP initiator), it is a useful method for preparing polymer-grafted materials (Siegwart & Matyjaszewski, 2012).

Already other research groups have worked in the production of polysaccharide macroinitiators to be employed in the ATRP process. For example, Meng et al. developed a cellulose based macroinitiator for the ATRP process of methyl methacrylate and styrene (Meng et al., 2009).

The aim of this work was to demonstrate that it is possible to employ ATRP as "growing from" technique to obtain HA derivatives with a narrow and controlled molecular weight distribution, both characteristics very important for a potential biomedical or pharmaceutical use of these materials.

The synthesis has been performed by using two subsequent steps. In the first step, two macroinitiators have been obtained by the conjugation of a proper number of 2-bromo-2-methylpropionic acid (BMP) to hyaluronic acid (as tetrabutyl ammonium salt: HA–TBA) or to ethylenediamino derivative of HA (HA–TBA–EDA). In the second step, HA–TBA–BMP and HA–TBA–EDA–BMP copolymers have been used as "multi-functional macroinitiators" for the polymerization via ATRP of poly(ethylene glycol) methacrylate (PEGMA), butyl methacrylate (BUTMA) or N-isopropylacrylamide (NIPAM) chosen as model molecules carrying vinyl groups affordable to the polymerization process and with hydrophilic (for PEGMA) or hydrophobic (for BUTMA and NIPAM) properties. Spectroscopic analysis and size exclusion chromatography have been used to verify the success of ATRP process in the production of new brush copolymers named as HA–BMP–pPEGMA, HA–BMP–pBUTMA, HA–BMP–pNIPAM, HA–EDA–BMP–pPEGMA, HA–EDA–BMP–pBUTMA and HA–EDA–BMP–pNIPAM that could be used as starting materials for preparing drug delivery systems or scaffolds for tissue engineering.

2. Experimental

2.1. Materials and methods

All reagents were of analytical grade unless otherwise stated.

Dimethylsufoxide (DMSO), acetone, 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride (EDC), N-hydroxysuccinimide (NHS), bis(4-nitrophenyl) carbonate (4-NPBC), tetrabutylammonium hydroxide (TBA–OH), diethylamine (DEA), 2-bromo-2-methylpropionic acid (BMP), poly(ethylene glycol) methacrylate (PEGMA) with a molecular weight of 360 Da, butyl methacrylate (BUTMA), N-isopropylacrylamide (NIPAM), copper bromide [Cu(I)Br], 2,2′-bipyridyl (Bpy), deuterium oxide (D_2O), dimethyl sulfoxide-d_6 (DMSO d_6), N,N-dimethylformamide-d_7 ($DMFd_7$) and chloroform-d ($CDCl_3$) were purchased from Sigma–Aldrich.

Hyaluronic acid (HA) having a low weight–average molecular weight was prepared by acidic degradation as reported by Shu et al. (Shu, Liu, Luo, Roberts, & Prestwich, 2002) starting from a biotechnological HA sodium salt, MW 1500 kDa that has been a generous gift from Novagenit s.r.l. (Italy). Briefly, 2 g of HA were dissolved in 200 ml of twice distilled water and the solution was kept in an orbital shaker incubator at 37 °C overnight. After this time, 4 ml of HCl 37% (w/v) were added and the solution was stirred with a blade stirrer for 5 min at 350 rpm. The solution was kept in orbital shaker incubator at 37 °C for other 24 h then the pH was adjusted to 7 with NaOH 1N. The so obtained solution was dialyzed against water for 5 days and the product was recovered by freeze drying.

Since HA is not soluble in organic solvents, the tetrabutylammonium salt of HA (HA–TBA) was produced as described in our previous work (Palumbo, Pitarresi, Mandracchia, Tripodo, & Giammona, 2006).

Hydroquinone, the stabilizing agent used in the commercial available PEGMA and BUTMA, was eliminated through basic activated aluminum oxide (Fluka) column.

Weight–average molecular weight (Mw) and polydispersity index (Mw/Mn) of starting HA and its derivatives here prepared were determined by a SEC apparatus equipped with a pump system (Waters 600, Mildford, MA, USA), a Universal column (particle size 5 μm) and a 410 differential refractometer (DRI) as a concentration detector (Waters 2410, Mildford, MA, USA).

Employed conditions were: 200 mM phosphate buffer (pH6.5):MeOH 90:10 (v/v) as a mobile phase, 36 ± 0.1 °C, flow rate 0.6 ml/min. The calibration curve was determined by using Pullulan standards from Hyalose (USA) as reported by Ferguson et al. (Ferguson, Alshame, & Thomas, 2010).

FT-IR spectra were recorded as pellets in KBr in the range 4000-400 cm^{-1}, by using a PerkinElmer 1720 Fourier Transform Spectrophotometer with a resolution of 1 cm^{-1}; each spectrum was recorded after 100 scans.

^1H NMR and Heteronuclear Multiple Quantum Coherence (HMQC) spectra were obtained by dissolving the samples in D_2O, $CDCl_3$ or mixture D_2O/$DMSOd_6$ or D_2O/$DMFd_7$ with a Brucker AC-300 instrument.

2.2. Synthesis of hyaluronic acid-ethylenediamine (HA–TBA–EDA) derivative

HA–TBA–EDA was synthesized as reported in our previous work (Palumbo et al., 2012).

Briefly, 500 mg of HA–TBA was dissolved in 45 ml of anhydrous DMSO then 121 mg of 4-NPBC, dissolved in 3 ml of DMSO, was added dropwise to the HA–TBA solution at 40 °C (molar ratio between 4-NPBC and HA–TBA equal to 0.50). The solution was left at the same temperature for 4 h. After this time, ethylenediamine (EDA) (molar ratio equal to 10 respect to the moles of 4-NPBC) was added and the solution was left at 40 °C for 3 h. The obtained HA–EDA–TBA derivative was precipitated in an excess of acetone then washed in the same solvent and dried under vacuum. The derivatization degree in terms of moles of EDA linked to HA (DD_{EDA}%), calculated by ^1H NMR analysis, was 50 mol%.

2.3. Synthesis of HA-based macroinitiators

The synthesis of HA–TBA–BMP and HA–TBA–EDA–BMP macroinitiators was performed as follows: 30 mg BMP were dissolved in anhydrous DMSO and, in order to activate the carboxyl group, 20 mg of EDC and 11.5 mg of NHS were added (molar ratio between EDC/NHS and BMP equal to 1). The obtained solution was stirred at 37 °C overnight then added to a 1% (w/v) solution of HA–TBA or HA–TBA–EDA in anhydrous DMSO, in the presence of DEA as a catalyst (molar ratio between DEA and primary hydroxyl groups of HA–TBA or amino groups of HA–TBA–EDA equal to 1). It was used a molar ratio between BMP–NHS and primary hydroxyl