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Hybrid injectable platforms for the in situ delivery of therapeutic ions from mesoporous glasses

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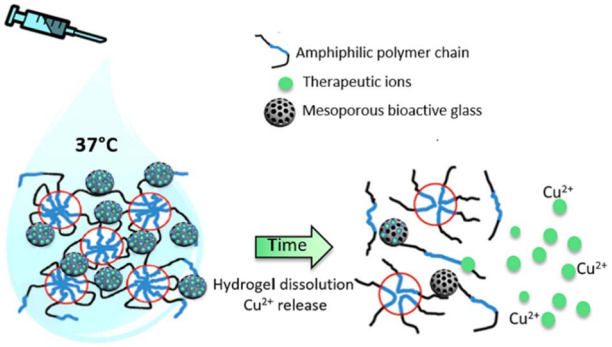
HIGHLIGHTS

- Cu-containing MBGs (Cu-MBG) synthesised as micro and nanospheres.
- Cu-MBGs incorporated into an injectable polyurethane hydrogel.
- Sustained and prolonged release of Cu^{2+} ions from incorporated Cu-MBGs.
- Hybrid injectable formulations for *in situ* delivery of therapeutic ions.

GRAPHICAL ABSTRACT



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ABSTRACT

Copper-containing bioactive glasses (Cu-MBGs) are attracting increasing interest as multifunctional agents for hard and soft tissue healing due to the ability of released copper ions to stimulate osteogenesis as well as angiogenesis and to impart anti-bacterial properties. The conjugation of these nanomaterials with a vehicle phase based on thermosensitive hydrogels represents an effective strategy to design non-invasive injectable devices for the *in situ* delivery of therapeutic ions from MBGs.

In this contribution, Cu-containing MBGs were prepared by an aerosol-assisted spray-drying method (MBG_Cu 2%_SD) in the form of microspheres (surface area of *ca* 220 $m^2 g^{-1}$) and through a sol-gel synthesis (MBG_Cu 2% _SG) in the form of spheroidal nanoparticles (surface area above 700 $m^2 g^{-1}$). Both Cu-containing samples were able to release copper ions, although with different rates and percentage release. MBG_Cu 2%_SG released the total incorporated amount of Cu ions with a faster kinetics compared to MBG_Cu 2%_SD, that released approximately the 60% of copper.

Cu-MBGs were incorporated with a final concentration of 20 mg/mL into a thermosensitive sol-gel system consisting of a novel amphiphilic poly(ether urethane) based on a commercialy available Poloxamer 407 (P407), with improved gelation ability, mechanical strength and stability in aqueous solution with respect to native P407. Cu-MBG-loaded hydrogels were characterised in terms of sol-to-gel transition temperature and time, injectability and stability in aqueous environment at 37 °C. The hybrid formulations showed fast gelation in physiological conditions (1 mL underwent complete sol-to-gel transition within 3–5 min at 37 °C) and injectability in a wide range of temperatures (5–37 °C) through different needles (inner diameter in the range 0.6–1.6 mm).

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When embedded into the hydrogel, Cu-MBGs retained their release properties, showing a sustained delivery of Cu^{2+} along 14 days.

1. Introduction

The use of organized mesoporous materials continues to gain interest in a wide range of biomedical applications due to their ability to store and release drugs/biomolecules and to the possibility to impart stimuli-responsive and/or targeting properties through surface functionalization [1–3].

In the field of advanced bioceramics, templated mesoporous glasses, which combine the textural features of ordered mesoporous matrices, i.e. very high exposed surface area and pore volume, with the properties of conventional bioactive sol-gel glasses, have shown excellent behaviour as systems for bone-tissue regeneration [4–6]. More recently, with the aim to develop a smart and versatile technology platform for highly targeted therapies for pathological tissues (hard and soft), the composition of mesoporous bioactive glasses (MBGs), conventionally synthesized in the systems SiO_2-CaO or SiO_2-CaO-P_2O_5, has been enriched with controlled amount of therapeutic elements, as Ag^+, Sr^{2+}, Cu^{2+} and Co^{2+}, whose release is able to impart specific biological functions, including anti-bacterial activity, as well as stimulation of osteogenesis and angiogenesis [7–11]. In particular, several studies have extensively proven the ability of released copper ions to enhance bone regeneration and vascularization, as well as wound healing [12,13]. Very recently, Wu and co-workers finely demonstrated the role of Cu^{2+} ions released from mesoporous silica nanospheres in inducing osteogenic/angiogenic response while suppressing osteo-clastogenic activity [14]. The sustained release of copper ions from MBGs can also act as an effective anti-microbial agent against both bacterial growth and biofilm formation/dispersion, providing a true alternative to traditional antibiotic systemic therapies [15]. Furthermore, the release of therapeutic ions can be synergistically combined with the delivery of pharmaceutical agents or growth factors incorporated into the internal cavities or adsorbed/grafted on the external surface.

The resulting multifunctional nanocarriers are ideal candidates for the development of novel clinical devices able to simultaneously target all the causes, often mutually interlocked, leading to compromised tissue healing. To this aim, the conjugation of these carriers with a vehicle phase based on thermosensitive hydrogels, in which an aqueous solution undergoes a sol to gel transition in physiological conditions (37 °C), represents an effective strategy to design non-invasive devices that can be injected into the pathological site and, after gelling *in situ*, will result in an ion/drug depot for prolonged and localised release. Recently, the feasibility of this approach has been proven for the preparation of an anticancer depot system based on thermosensitive Pluronic F127 hydrogel carrying doxorubicin-loaded mesoporous silica nanoparticles [16]. Pluronic F127, also coded as Poloxamer 407, is a poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) (PEO-PPO-PEO) triblock copolymer extensively used in biomedical applications [17]. However, Pluronic F127-based hydrogels suffer of poor stability in aqueous environment, starting to dissolve few minutes after injection and thus do not allow a prolonged and sustained release overtime [18,19]. One of the most commonly investigated approaches to overcome this limitation consists in chain extending Pluronic F127 through the chemistry of polyurethanes (PURs), which, amongst other things, also allows the introduction of specific moieties for further functionalization, targeted degradation or specific cell behaviour along the polymer backbone. As a consequence of the high chemical versatility that characterizes this class of polymeric materials, a wide range of PUR compositions has been designed and thoroughly investigated for different applications in the fields of regenerative medicine and pharmaceutics, ranging from implantable biostable devices to biodegradable scaffolds and delivery systems [18–27]. Hence, PURs act as a flexible platform of materials able to fit the requirements imposed by the final application. In particular, PUR composition can be opportunely selected based on the required mechanical properties of the resulting PUR-based device: rigid polyurethane scaffolds are suitable for bone tissue replacement [28–30], while soft polyurethane constructs better fit with the regeneration of soft tissues (i.e. cardiac, vascular and nerve tissues) [25,31,32].

For some applications, such as filling of restricted, non load bearing defect sites, injectable PUR-based hydrogels, able to perfectly take the shape of the defect cavity prior to their complete gelation, have been successfully developed to allow easy administration and minimally invasive injection [33].

In this regard, Boffito et al. [18] recently reported the synthesis of a novel amphiphilic poly(ether urethane) (PEU) based on Poloxamer 407, with improved gelation ability, mechanical strength and stability compared to the commercially available Poloxamer 407, showing promising features for application in tissue engineering/regenerative medicine and drug release as *in situ* injectable delivery systems. In the literature, injectable PURs developed for bone tissue engineering have been conjugated with an inorganic phase, such as tricalcium phosphate or mineralized bone particles, with the aim to improve the mechanical properties and confer osteoconductivity to the final hybrid constructs [31,34].

In this study, with the aim to prepare an injectable hybrid formulation able to release *in situ* therapeutic ions from MBG particles, a Poloxamer 407-based PEU has been synthesized according to a previously published method [20] and its aqueous solutions have been loaded with MBGs belonging to the SiO_2-CaO-CuO system to design a hybrid thermosensitive MBG-PEU platform.

Copper-containing MBGs have been prepared in the form of micro- and nano-spheres by following two synthesis approaches in order to evaluate the effect of particle size and size distribution on the resulting formulation properties. The obtained MBG-PEU systems have been investigated in terms of sol-to-gel transition temperatures and time, injectability and stability in aqueous environment at 37 °C to mimic the real working conditions of the developed systems. The release profiles of Cu^{2+} ions from the glass matrices alone and upon loading into the hydrogel have been assessed and the obtained kinetics compared.

2. Materials and methods

2.1. Preparation of Cu-containing MBGs

Copper-loaded MBGs were prepared through two different synthesis approaches in order to obtain particles with different size and structural parameters (i.e. specific surface area, pore size).

2.1.1. Preparation of Cu-containing MBG nanoparticles by sol-gel synthesis

The first procedure is a base-catalysed sol-gel synthesis, as reported by Wu et al. [35] to produce mesoporous calcium-silicate nanoparticles, where copper replaces part of calcium. In particular, MBG with 2% molar percentage of Cu (molar ratio Cu/Ca/Si = 2/13/85, named hereafter as MBG_Cu 2%_SG) was prepared as follows: 6.6 g cetyltrimethylammonium bromide (CTAB ≥ 98%, Sigma Aldrich, Italy) and 12 mL NH_4OH (Ammonium hydroxide solution, Sigma Aldrich, Italy) were dissolved in 600 mL of double distilled water (ddH_2O) under stirring for 30 min. Then, 30 mL tetraethyl orthosilicate (TEOS, Tetraethyl orthosilicate, Sigma Aldrich, Italy), 4.888 g of calcium nitrate tetrahydrate ($Ca(NO_3)_2{\cdot}4H_2O$, 99%, Sigma Aldrich, Italy) and 0.428 g of