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Is there a cause-and-effect relationship between physicochemical properties and cell behavior of alginate-based hydrogel obtained after sterilization?

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ABSTRACT

Alginate-based hydrogel scaffolds are widely used in the field of cartilage regeneration and repair. If the effect of autoclaving on the alginate powder is well known, it is not the same for the possible effects of the sterilization UV treatment on the properties of the hydrogel after polymerization. To select an effective sterilization treatment of alginate-based materials, one must find what are inter-relationship between the characteristics (chemical, physical and mechanical) of alginate-based hydrogel during sterilization, and what consequences have affected on cell behavior. In this study, we investigated the influence of UV sterilization treatments (UV-1 and UV-2: 25 and 50 min, respectively) and autoclaving to obtain alginate (Alg)/hyaluronic acid (HA) hydrogel, as well as further evaluated the relationship between physicochemical properties and cell behavior of Alg/HA hydrogel after UVs and autoclaving. The physicochemical properties of this mixture at the powder or polymerized states were analyzed using ATR-FTIR, HPLC-SEC, rheological, indentation testing and sterility testing. The cell behaviors of hydrogels were evaluated by cell viability and proliferation, and chondrogenic differentiation. The effects of treatment parameters and their correlation with the others characteristics were determined statistically by Principal Component Analysis (PCA). In this study, we have shown that the cell behavior in alginate-based hydrogels was not only regulated by physicochemical properties (as molar mass or/and viscosity), but also associated with the controlling of sterilization time. It can provide a basis for choosing an effective method of sterilization, which can keep the mechanical or physical-chemical properties of Alg-based hydrogel scaffold and maintain its cytocompatibility and its ability to induce chondrogenesis from mesenchymal stem cells.

1. Introduction

Articular cartilage has poor ability to self-repair, and damage due to tissue lesion, trauma, or natural degeneration. In order to repair the damaged cartilage, many researchers have promoted alginate-based scaffold to use in field of tissue-engineered cartilage due to it is easily to construct the hydrogels which support cell encapsulation and culture. Several studies have shown that alginate-based scaffold can promote mesenchymal stem cells (MSC) differentiation to chondrocyte phenotype, and synthesis of cartilage-specific matrix (Zhao et al., 2013; Van Vlierberghe et al., 2011; Sun and Tan et al., 2013; Xu et al., 2008; Reppel et al., 2005). Meanwhile, sterilized alginate-based composite powder is imposed. So the effective sterilization methods play an important role in the application of biomedical that not only eliminate the risk of infection, but also maintain structure, function and use of materials (Ahmed et al., 2013). However, it is reported that alginate is difficult to sterilize and to handle (Puppi et al., 2010). Even if Draget et al. (1990) produced sterilized alginate by filtration *via* an appropriate submicron filter, it is not very convenient for highly viscous of alginate hydrogel. Generally, moist heat (Hu et al., 2014; Ofori-Kwakye and Martin, 2005), autoclaving (Leo et al., 1990; Vandenbossche et al., 1993; Ofori-Kwakye and Martin, 2005), ethylene oxide (Leo et al., 1990), gamma-irradiation (Lee et al., 2003) and UV (Mao et al., 2012) are used to sterilize the alginate-powder, but for both of them lead to the chain scission and hemolysis of alginate due to the high pressure and temperature which the process of it is called de-polymerization.

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However, among all these sterilization process, there is no statistic analysis that studies the inter-relationship between the physicochemical characteristics of alginate hydrogel after sterilization and cell behavior.

Alginate (Alg) could be obtained from brown algae and some soil bacteria. This is a linear polysaccharide containing blocks of (1,4)-linked β-D-mannuronic acid (M units) and α-L-guluronic acid (G units) residues which are believed to take part in intermolecular cross-linking with Ca^{2+} to form hydrogels (Lee and Mooney et al., 2012). The structure of alginate hydrogel is similar to the glycosaminoglycan (GAG), which is long unbranched polysaccharides makes up the cartilage (Wang et al., 2009). Several studies reported that alginate-based hydrogel have a good biocompatibility, non-immunogenic and chondrogenic differentiation, when MSC were seeded into hydrogel (Xu et al., 2008; Follin et al., 2015; Guo et al., 2014; Du et al., 2016). Similarly, hyaluronic acid (HA) is a glycosaminoglycan consisting of D-glucuronic acid and D-N-acetylglucosamine units. It is an important component of proteoglycan organization which composed cartilage tissue. Meanwhile, it can contribute to the function of the tissue itself (Collins et al., 2013). It is considered as a chondrogenesis inductor of MSC (Chung and Burdick, 2009). Borzacchiello et al. (2015) made the HA hydrogel through the cross-linking HA molecules with divinyl sulfone (DVS) and have shown it has a good biocompatibility, mechanical and injectability properties suited to biomedical application. So, both alginate and HA natural polymers exhibit properties of biocompatibility, biodegradability, non-immunogenicity and low cost. They can be an attractive hydrogel candidate for cartilage tissue engineering. In our work, we planned to combine the alginate with hyaluronic acid as raw materials to form hydrogel scaffold. Even if the effects of autoclaving on alginate powder properties are known, very few is know about the effect of UV as sterilization treatments on alginate-based composite powder properties and on its capacity to maintain chondrogenic differentiation *in vitro*. Meanwhile, our ultimate goal was to estimate the correlation between physicochemical properties and cell behavior of alginate-based hydrogel during sterilization.

2. Materials and methods

2.1. Sterilization treatments of powder

The powder of 1.5% (m/v) alginate (medium viscosity, Sigma-Aldrich, France) and hyaluronic acid (Acros organics, USA) (ratio 4:1) was sterilized by UV-1 treatment (ultraviolet during 25 min), UV-2 treatment (ultraviolet during 25 min and was turned over during 25 min) (Ultraviolet lamp=2×15 w, wavelength=254 nm, Distance=30 cm, Vilber lourmat, France), autoclaving at 121 °C for 30 min (Advantage-Lab, AL02-07-100, France), non-sterilized (without any treatments for the powder, as to be control). Then, they were homogeneously dissolved in 0.9% sodium chloride (Merck, Germany) to form gel solution by constant stirring in a sterile glass tube.

2.2. Hydrogel scaffold construct, cell culture and chondrogenic differentiation

To prepare gel solution as we described above. A syringe was used to absorb the gel solution before being dropped in a 102 mmol/L $CaCl_2$ (Sigma-Aldrich, France) solution for 10 min, forming spherical beads (Alg/HA) completely. Then the hydrogel beads were washed three times by 0.9% NaCl solution, which was further studied. In addition, we used the spraying method to make the hydrogel scaffold which was previously described with cells (Tritz et al., 2010; Tritz-Schiavi et al., 2010). The spraying system is composed of an airbrush working with a compressor (Mjahed et al., 2008). The spraying bottle containing the gel solution was connected to the airbrush, and then solution was sprayed on a sterile glass plate with the spraying pressure is equal to 0.9 bar, which was quickly and horizontally putted into a bath of 102 mM $CaCl_2$ for 10 min, forming the 3D-Alg/HA hydrogel scaffold. And then scaffolds were washed two times with a 0.9% NaCl solution. These scaffold without cells were kept in 1 mM of $CaCl_2$ at room temperature before Attenuated Total Reflection Fourier Transform Infrared (ATR-FTIR) analysis and mechanical testing.

Wharton's jelly mesenchymal stem cells (WJ-MSC) were isolated by the explant methods from the umbilical cord as previously described (Reppel et al., 2014). Briefly, human umbilical cords were collected after patents' informed consent which was regarded as surgical waste, as well as on the basis of the guidelines for the care and use of an ethic committee of Nancy Hospital. Umbilical cords were washed with 70% ethanol and Hanks' balanced salt solution (HBSS). The umbilical cord vessels were removed and Wharton's jelly aseptically cut into very small pieces which were cultured in a six-well plate with complete medium (Alpha Modified Eagle Medium, α-MEM, Lonza, belgium) with 10% Fetal bovine serum (Sigma-Aldrich, France), 100 IU/mL penicillin (Gibco, France), 100 µg/mL streptomycin (Gibco, France), 2 mM L-glucose (Sigma-Aldrich, France), and 2.5 µg/mL amphotericin B (Gibco, France). They were incubated at 37 °C in 5% CO_2, 5% O_2 incubator. After 7 days, cells have migrated from Wharton's jelly and adhered on plate. Then, Wharton's jelly pieces have been removed and culture medium was changed twice a week until cell subconfluence (80%). After 2 weeks, WJ-MSC were harvested with 0.25% Trypsin-EDTA (Sigma-Aldrich, France) and seeded in a new culture flask with a density 1000 cells/cm². Media were changed three times weekly. This process was repeated until culture up to the third passage. For viability, proliferation and chondrogenic differentiation analysis, WJ-MSC were encapsulated into Alg/HA hydrogel beads, manufactured as previously described (Wang et al., 2008). Scaffolds were cultivated in 6-well plates with induced chondrogenic culture medium which contained DMEM-high glucose (Gibco, Grand Island, NY) supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 IU/mL penicillin, 100 µg/mL streptomycin, 2.5 µg/mL amphotericin B, 1 mM $CaCl_2$, 0.1 µM dexamethasone (Sigma-Aldrich, France), 50 µg/mL ascorbate-2-phosphate (Sigma-Aldrich, France), 100 µg/mL sodium pyruvate (Sigma-Aldrich, France), and 40 µg/mL L-proline (Sigma-Aldrich, France). Scaffolds were incubated at 37 °C in a humidified incubator with 5% CO_2, 5% O_2 for 30 days. The differentiation medium was changed three times weekly.

2.3. Chemical properties of hydrogel scaffold

2.3.1. Fourier Transform infrared (FTIR) spectroscopy

Attenuated Total Reflection Fourier Transform Infrared (ATR-FTIR) spectra were recorded on a Fourier transform infrared spectrometer (170SX, Nicolet Co., Madison, WI, USA) to analyse the chemistry of prepared 3D-Alg/HA hydrogel scaffolds during these sterilization methods. Each sample was dried on vacuum desiccator at 60 °C for 24 h, then cut into small pieces and moved onto ATR crystal, which were measured with a FTIR spectrometer. The infrared spectra of the raw materials (dry alginate and hyaluronic acid powders) were also respectively measured with a FTIR spectrometer; the spectra were collected over range of 4000–500 cm^{-1}.

2.3.2. Molecular weights evolution

Size Exclusion Chromatography (SEC) was performed at room temperature to quantify some possible degradation of Alg/HA during treatments. SEC was performed using a Waters HPLC pump equipped with a serial set of SP 806, 805 and 804 OH pack columns (Shodex). Elution was carried out with 0.1 M $NaNO_3$ containing NaN_3 as a bactericide (0.4 g/L) at 0.7 mL/min, and was monitored by Multi Angle Laser Light Scattering detector (MALLS Mini Damn, Wyatt) and differential refractometry (Waters 410) dual detection.

Solution (2 mg/mL) were prepared by dissolution in the same eluent and left under vigorous stirring for 24 h. Filtration of solutions