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# Poly(amido-amine)-based hydrogels with tailored mechanical properties and degradation rates for tissue engineering

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ABSTRACT

Poly(amido-amine) (PAA) hydrogels containing the 2,2-bisacrylamidoacetic acid-4-amminobutyl guanidine monomeric unit have a known ability to enhance cellular adhesion by interacting with the arginin–glycin–aspartic acid (RGD)-binding αVβ3 integrin, expressed by a wide number of cell types. Scientific interest in this class of materials has traditionally been hampered by their poor mechanical properties and restricted range of degradation rate. Here we present the design of novel biocompatible, RGD-mimic PAA-based hydrogels with wide and tunable degradation rates as well as improved mechanical and biological properties for biomedical applications. This is achieved by radical polymerization of acrylamide-terminated PAA oligomers in both the presence and absence of 2-hydroxyethylmethacrylate. The degradation rate is found to be precisely tunable by adjusting the PAA oligomer molecular weight and acrylic co-monomer concentration in the starting reaction mixture. Cell adhesion and proliferation tests on Madin–Darby canine kidney epithelial cells show that PAA-based hydrogels have the capacity to promote cell adhesion up to 200% compared to the control. Mechanical tests show higher compressive strength of acrylic chain containing hydrogels compared to traditional PAA hydrogels.



## 1. Introduction

Hydrogels are defined as three-dimensional (3-D) networks of hydrophilic polymers that are able to absorb up to hundreds of times their dry weight in water, thus acquiring biomechanical properties similar to the extracellular matrix (ECM) and allowing cells to adhere, proliferate and differentiate onto their surface. Several classes of hydrogels are characterized by excellent biocompatibility and minimal inflammatory reaction and tissue damage, which make them attractive materials for tissue engineering and regenerative medicine [1]. Hydrogels based on synthetic polymers, thanks to their versatility and low costs compared with natural biomaterials, have been successfully employed in a variety of biomedical applications, ranging from ophthalmic and vascular prostheses to drug delivery and soft-tissue replacement [2–4].

In particular, hydrogels based on poly(2-hydroxyethylmethacrylate) (PHEMA) have shown to hold several advantages over other synthetic polymers [5]. Indeed, they display excellent biocompatibility in vivo and can be fabricated with different architectures and mechanical properties similar to natural tissue [6–9]. Even though PHEMA homopolymers cannot be degraded enzymatically or by acidic/alkaline solutions, copolymers containing HEMA were found to be biodegradable [8].

Another promising class of synthetic polymers for tissue engineering application are poly(amido-amine)s (PAAs) [10]. They are obtained by Michael-type addition of bis-acrylamides to primary amines and/or secondary diamines, under mild conditions. Different bioactive molecules can be incorporated into the PAA's backbone by covalent attachment during the synthetic process [11–13]. PAA-based hydrogels show good biocompatibility and are extremely versatile, being easily modifiable by introducing different co-monomers that carry additional chemical functions such as carboxylic acids, thiols and amino groups [14–20].

Biological properties of synthetic materials can be further improved through surface/bulk modification with bioactive functional groups able to interact specifically with cell receptors [21,22]. For example, cell adhesion has been improved by introducing the tripeptide arginin–glycin–aspartic acid (RGD), which is

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specifically recognized by proteins involved in cell adhesion, such as fibronectin, laminin and vitronectin [23,24]. However, despite their good biological properties and low immunological reaction risk, the manufacture of RGD-modified hydrogels requires a complex synthetic procedure and high production costs [25].

To overcome these hurdles, Tanahashi et al. modified the surface of poly(propylene fumarate-co-ethylene glycol) hydrogels with 4-aminobutyl guanidine (ABG) units. Interestingly, they demonstrated that the increased fibroblast cell adhesion was related to the RGD-mimic structure of the guanidine side groups [26].

The introduction of ABG units in a cross-linked amphoteric PAA hydrogel, as demonstrated by Ferruti et al., resulted in increased fibroblast cell adhesion [15]. However, PAA hydrogels displayed some drawbacks such as non-controllable degradation rate, scarce mechanical properties and high risk of ruptures during hydrogel washing due to osmotic pressure [14–15,27].

Since it has been demonstrated that the ABG residue in PAA hydrogels maintains cell-adhesion-promoting ability even when copolymerized with other monomers, we supposed that, by introducing HEMA as acrylic co-monomer in the 3-D network, it would

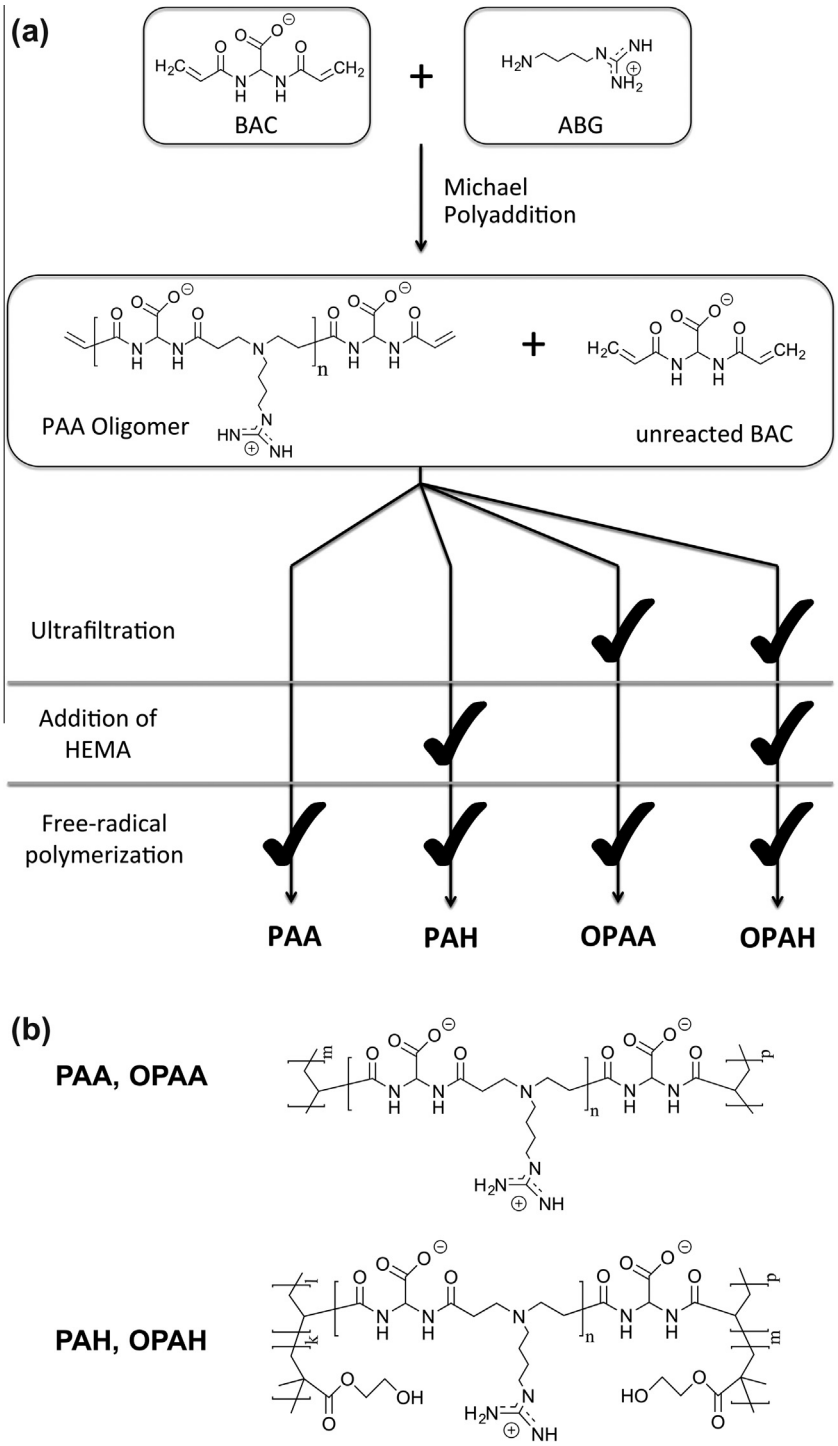


**Fig. 1.** (a) Synthetic scheme of PAA, OPAA, PAH and OPAH hydrogel series; (b) chemical structure of the synthesized hydrogels.