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Conductive hydrogels with tailored bioactivity for implantable electrode coatings

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ABSTRACT

The development of high-resolution neuroprosthetics has driven the need for better electrode materials. Approaches to achieve both electrical and mechanical improvements have included the development of hydrogel and conducting polymer composites. However, these composites have limited biological interaction, as they are often composed of synthetic polymers or non-ideal biological polymers, which lack the required elements for biorecognition. This study explores the covalent incorporation of bioactive molecules within a conducting hydrogel (CH). The CH was formed from the biosynthetic co-hydrogel poly(vinyl alcohol)–heparin and the conductive polymer (CP), poly(3,4-ethylene dioxythiophene). Adhesive biomolecules sericin and gelatin were covalently incorporated via methacrylate crosslinking within the CH. Electrical properties of the bioactive CH were assessed, and it was shown that the polar biomolecules improved charge transfer. The bioactivity of heparin within the hybrid assessed by examining stimulation of B-lymphocyte (BaF3) proliferation showed that bioactivity was retained after electropolymerization of the CP through the hydrogel. Similarly, incorporation of sericin and gelatin in the CH promoted neural cell adhesion and proliferation, with only small percentages ($\leqslant$2 wt.%) required to achieve optimal results. Sericin provided the best support for the outgrowth of neural processes, and 1 wt.% was sufficient to facilitate adhesion and differentiation of neurons. The drug delivery capability of CH was shown through incorporation of nerve growth factor during polymer fabrication. NGF was delivered to the target cells, resulting in outgrowth of neural processes. The CH system is a flexible technology platform, which can be tailored to covalently incorporate bioactive protein sequences and deliver mobile water-soluble drug molecules.

1. Introduction

Conducting polymers (CP) such as poly(ethylene dioxythiophene) (PEDOT) have been investigated for biosensor and neuroprosthetic electrode applications because of their superior electrical properties in comparison with conventional metals such as gold and platinum [1–3]. Although CP have demonstrated high charge transfer capability, studies indicate that this performance is not maintained across chronic implantation time-frames, owing to limited mechanical stability, including delamination and loss of friable material at the neural interface [4,5]. One strategy that has been extensively explored to improve the long-term performance of CP films is the incorporation of biofunctional molecules such as proteins and peptides within the CP matrices [6–11]. While several molecules have been shown to improve initial cell attachment or promote cell differentiation, these relatively large molecules inhibit efficient electropolymerization of the CP, resulting in brittle, friable polymers with poor electroactivity, exacerbating problems with chronic mechanical stability [11]. To address the limitations of CP a number of composite polymer systems have been proposed, with a strong focus on composites of CP and hydrogels [2]. It has been shown that composite CP–hydrogels or conductive hydrogels (CH) can promote the formation of a more stable electrode material with the benefits of both polymer components, including electroactivity and drug delivery [12–14].

The hydrogel component of a CH provides a matrix with which the friable CP can be integrated, preventing the loss of material under the shear force conditions typically experienced within an implant environment. Several studies have explored the different types of CH composites, including CP-alginate [15], PEDOT-PAA (polyacrylic acid) [16], CP-PAMPS (poly(2-acryl-amido-2–2methyl-1- propanesulfonic acid)) [17] and CP-pHEMA (poly(2-hydroxyethyl methacrylate)) [18] and poly(vinyl alcohol) (PVA) [21]. These CH have been shown to have improved mechanical properties and biological compatibility, with the electroactivity of the CP preserved, and in some cases improved by polymerization throughout the

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hydrogel. Kim et al. recorded impedance values for polypyrrole (PPy) grown through alginate hydrogel at one order of magnitude lower than the impedance for homogeneous PPy [12]. It was proposed that increased conductivity was due to the resulting three-dimensional structure (3-D), which enabled charge transfer to occur across a substantially greater surface area [19].

A recent study by Green et al. [20] demonstrated that coating platinum electrodes with a hybrid of PEDOT and the biosynthetic co-hydrogel, poly(vinyl alcohol)–heparin (PVA-Hep), significantly reduced electrode stiffness in comparison with uncoated platinum or CP films alone, making it a closer mechanical match with neural tissue. The heparin was incorporated to prove the principle that a biological molecule covalently incorporated in a hydrogel could act as a dopant for the CP deposited throughout the hydrogel. While cell response was improved compared with the homogeneous hydrogel, cell attachment was reduced in comparison with the conventional CP, PEDOT doped with poly(styrene sulfonate) (PEDOT/PSS) [20]. As close contact with interfacing neurons is desired to reduce neural cell stimulation thresholds and preserve the interface without scar tissue ingrowth [3], this was considered suboptimal biological performance. This was attributed to the dominance of the hydrogel component conferring hydrophilicity and anti-fouling characteristics to the hybrid surface [21,22]. Additionally, it was recognized that heparin, while being a bioactive molecule, has no domains that promote cell attachment. Although it is well known that hybridization of CP and hydrogels can have a significant impact on CP physical and electrical properties [2,19], an optimal CH should enable tailoring of the material properties to specific applications. While the properties of the CP are largely uncontrollable when electrodeposition is used to promote polymerization, the hydrogel component is highly flexible. Therefore, this study examined the effect of manipulating the CH properties through variation of the hydrogel component.

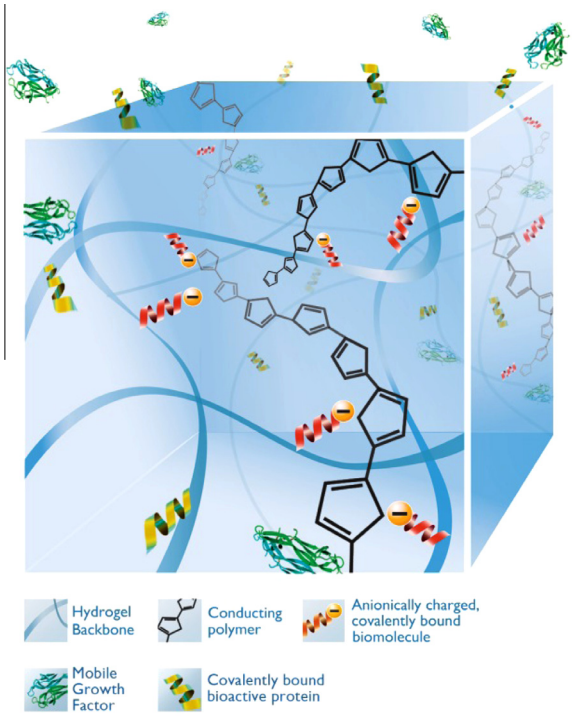

Fig. 1. Schematic of biofunctionalized CH, consisting of a conducting polymer component grown through a biosynthetic hydrogel, which comprises an anionically charged biological dopant, additional covalently bound proteins (potentially with cell attachment sequences) and mobile diffusible growth factors.

It was hypothesized that CH can accommodate incorporation of multiple biological polymers, both covalently bound and freely diffusible, as detailed in Fig. 1, to impart bioactivity without adversely affecting the physical and electrical properties. Furthermore, the addition of biological molecules with cell adhesive sequences can improve cell interactions with the resulting CH. Selection of the biological molecule for incorporation is the key to the function of the CH produced. Proteins with cellular adhesion sequences such as arginine-glycine-aspartic acid (RGD) include extracellular matrix proteins such as laminin [23], collagen [24] and its derivative gelatin [25]. Other naturally occurring proteins such as sericin, which acts as a "glue" for silk fibroins and is produced by silkworms, can promote cell attachment, proliferation and differentiation [26–29]. Glycosaminoglycans such as chondroitin sulfate [30] and heparin [31] can confer other functionality, such as growth factor presentation and anticoagulation [31,32]. In this study gelatin, sericin and heparin were chosen for covalent incorporation into the CH. This choice was largely influenced by the availability of bulk quantities and the ability to process these molecules for covalent incorporation within the hydrogel matrix. Covalent incorporation of biomolecules within PVA hydrogels can be facilitated by methacrylate crosslinking, which requires modification of the biomolecule using established methods [31]. The minimum amount of product required to functionalize molecules via methacrylation is ~1 g, with a 60–70% yield. Purifying laminin, a more common neural interfacing biomolecule, in the required quantities is inefficient and unlikely to be translatable into the clinical environment. While collagen has also been investigated with respect to encouraging neural cell attachment to bioelectrodes, gelatin contains the same adhesive sequences, but in a less bulky molecule. Heparin, sericin and gelatin are all compatible with this synthesis and can be processed with high efficiency [29,31]. Additionally, they all have specific biofunctionality that can be used to improve the neural interface.

Previous studies by Prasad et al. and Martens et al. demonstrated that gelatin [33] and sericin [34] supported PC12 adhesion and differentiation, and it is expected that these biomolecules will exhibit similar properties when they are incorporated within the CH system. It has also been shown that changing the bulk mechanical property of the hydrogel (from a 20 wt.% to a 30 wt.% gel) component of a CH does not significantly alter the hybrid mechanical modulus [20]. Additionally, studies by Lim et al. have shown that addition of 1–5 wt.% protein within 20 wt.% PVA-based hydrogels has no impact on the homogeneous hydrogel modulus or other physical properties such as mass loss and swelling [35]. Therefore, to preserve the mechanical modulus, proteins will be incorporated at low percentages, ranging between 1 and 5 wt.%. While much is known regarding the incorporation of biologics into hydrogels, there is minimal information on how the bioactivity and accessibility of these molecules is affected by the addition of a relatively stiff, hydrophobic CP.

It is known that small ions are ideal for doping CP [36] and that larger biomolecules impart significant brittleness, which leads to coating failure [10,11], but the CH system can incorporate these larger biomolecules without loss of mechanical integrity. It is also known that biomolecules can be used to dope CP, with molecules such as hyaluronic acid and dexamethasone-phosphate being investigated in the literature [9,37,38]. Since sericin is a negatively charged biomolecule, it is anticipated that it can also act as a dopant for the CP component, thereby enhancing the PEDOT formation throughout the CH. Even though gelatin is overall a weakly positively charged molecule, it contains some negatively charged domains where PEDOT could potentially interact, contributing to the doping of the CP component. Therefore, it is anticipated that the addition of these biomolecules will not interfere with the formation of the CP throughout the hydrogel, and the resultant