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Fabrication and Preliminary Characterization of Bio-compatible Starch hydrogels

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Abstract: Collagen is essential for skin elasticity. In case of a wound, collagen synthesis is suppressed, only to be up-regulated later during wound healing. Hence, it was felt that the application of a bio-compatible hydrogel onto the affected area could accelerate this process of wound healing by providing a scaffold for fibroblasts to grow, apart from its applications in delivering cells and/or drugs. While Collagen, Chitosan have been used as substrates for hydrogels in the past, they have extraction protocols beyond our reach and will prove to be expensive. Starch was chosen over collagen due to its cost-effective nature and this material is known to be bio-compatible. Glutaraldehyde was used as the cross-linking agent due to its high reactivity. Different concentrations of components were tried to obtain an optimal hydrogel with the desirable characteristics of low weight, moderate elasticity (optimization of relative PVA concentration), swelling properties and shaping using molds of our choice. Surface properties of the membrane like pore size were studied using light microscopy and AFM. AFM data showed an uniform texture (with and without $NaHCO_3$). Swelling ratio was also studied and we demonstrated that the gels could be repeatedly hydrated and dehydrated and were sturdy and durable, especially at a larger hydrogel thickness, and temperature resistant. This approach can be taken as a cost-effective method to prepare a 0.5-1 mm starch hydrogel infused with anti-microbial drugs, and further use it as a wound dressing to speed up the healing process, subsequent to the optimization of the key variables like pore geometry, elastic properties as well as the use of natural cross-linkers, apart from its utility in cell-based applications.
Keywords: hydrogel; starch; wound dressing; drug delivery; swelling.

Introduction and Experimental:

Hydrogels (3D-crosslinked hydrophilic network (capable of absorbing and retaining water within their interstitial spaces) can be created by the linking of synthetic and/or natural polymers in a network) [1][2][3]. In this regard, starch is bio-functional, biocompatibile, inexpensive, abundant, biodegradable and non-toxic [4]. This study involves preparing a hyrdrogel membrane with starch: polyvinyl alcohol combination and glutaraldehyde-mediated cross-linking [5] [6].

Materials: Glutaraldehyde (GA), starch, 70% ethanol, 35% pure HCl, polyvinyl alcohol (Hot PVA, MW 14000), and MilliQ water were used for the experiment. GA reagent was prepared by mixing 0.1mL of GA with 2mL of 70% ethanol and 0.01mL HCl. Lab grade NaHCO3 were used as porogens.

Preparation of hydrogel: 10% and 20% (w/v) solutions of PVA were prepared in beakers by dissolving PVA in water and heating up to 80°C while stirring constantly. The solution was heated until homogenous, and the top layer of PVA was removed with a glass rod. 5% starch solution (w/v) was prepared by heating the solution at 100°C with constant stirring. 10mL of this solution was then added to 10mL (10%/20%) of the PVA solution and mixed to get a uniform mixture. To this, 2.11 mL of the GA reagent was added with constant stirring. The hydrogels were cast in molds and dried for around 2 days. They were washed with MilliQ water to remove the cross-linker and then re-dried at room temperature, and hydrated [5] [6].

Swelling studies: The dry hydrogels were immersed in MilliQ water, and allowed to hydrate/swell for 16 hours, to a constant weight. The equilibrium swelling ratio was calculated using the standard formula [7]

Characterization*:*The hydrogels were visualized and photographed under a light microscope at 4x, 10x and 40x magnifications. The pores geometry and uniformity were focused upon. Small regions of the hydrogels, were subjected to atomic force microscopy (AFM) [8] under contact/static mode, with scan areas of 2µm, 5µm and 10µm, to study the surface topography.

Results and Discussion:

Preparation of hydrogel: The hydrogel was prepared based on the aforementioned protocol. In this, we varied the concentration of the reagents to obtain optimum mixture and subsequently best results. One important observation made was that 20% PVA resulted in a grainy, clumpy texture and caused a lack of uniformity in the hydrogel. As a result we used 10% PVA.[5][6]

Swelling studies: The swelling studies observations can be found in Table 1. Given that we observed optimum pore size control with Sodium Bicarbonate, we used it as our primary porogen in the swelling studies. The concentration was varied and compared to a control. The swelling capacity of the membrane was found to reduce on application of porogen. However it showed an increase by increasing the porogen concentration. The hydration of the native sample can be seen in Figures 1 and 2. [7].

Characterisation: The hydrogel was characterized with Atomic Force Microscopy. The AFM imaging indicated a surface pattern with consistency or uniformity [Figure 3] [8]. Such membranes, can be evaluated for drug entrapment and release, wound dressings as well as scaffolds for cell-based applications, apart from studies to obviate the need to use a toxic cross-linker like glutaraldehyde. Additional characterization with the help of FTIR, tensile testing and scanning electron microscopy are currently under process and will help evaluate the membrane in a more comprehensive manner.

Table 1: Swelling study observations

S.N.	Sample	Weight of dry sample	Weight of hydrated sample	Swelling ratio
1.	With 0.05g NaHCO3	0.8g	1.27g	1.58
2.	With 0.1g NaHCO3	0.55g	1.14g	2.07g
3.	Plain, without salt	0.27g	0.69g	2.55

Figure 1: 10x magnification - dry hydrogel

Figure 2: 10x magnification - wet hydrogel

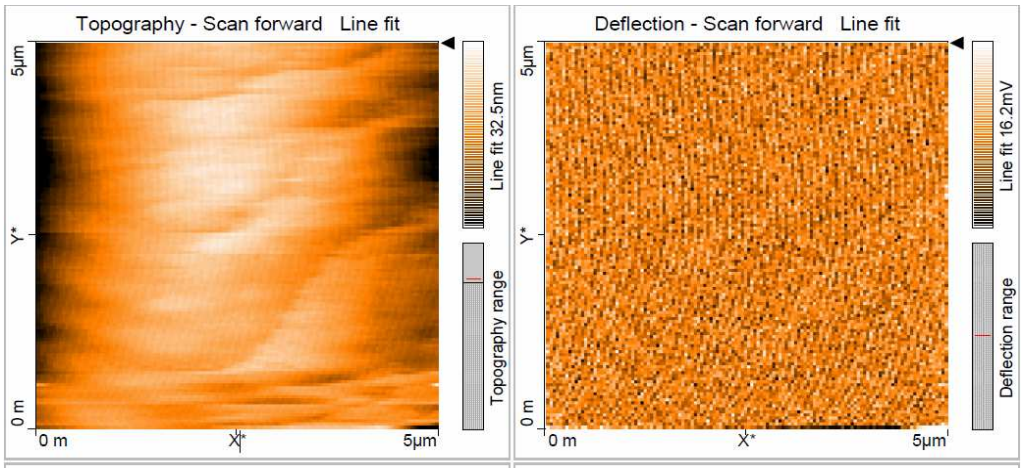

Figure 3: AFM imaging at 5µm scan area in contact/static mode

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