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Hydrogel Optimization Towards Fibroblast-friendly Biomimetic Coatings for Implantable Devices

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***Abstract*— In this paper we present our investigations related to the optimization of hydrogels for the coating/packaging of biomedical devices. In order for hydrogels to be a viable interface/packaging material, a number of conditions must be met. We outline the tailoring of the mechanical properties of a HEMA based hydrogel by exploiting the influence of individual hydrogel components to achieve these requirements. The water sorption, the elasticity and the porosity of various hydrogel materials were tested and the effects of the different hydrogel components was determined. These components include gelatin (used as a pore generator or porogen), alginate (to influence mechanical properties) and collagen (to improve cell adhesion). We also report the results of *in vitro* fibroblast testing on various hydrogel types.**

I. Introduction

In the continuous evolution of implantable biomedical microsystems [1], special attention is paid to the interface between the implantable microdevice and the body fluids and tissue. The material selected for the surface of an implant should be biocompatible thus avoiding the induction of unwanted bodily reactions [2], while maintaining the functionality of the biomedical microsystem.

For active implantable devices with a sensor monitoring a biopotential or some other tissue/fluid property of interest, one of the main long-term device failure mechanisms is the so called foreign body reaction (FBR) which is characterized by long term, low level inflammation and macrophage activation. This FBR results in a device that is completely isolated from the natural cellular environment by a collagenous avascular fibrous layer roughly 50-200µm thick [3-6]. In case a sensor is present, this thick fibrous layer will prevent correct sensing. Related to biocompatibility and another frequent cause of implant failure is biofilm formation due to bacteria. Bacteria can colonize the surface of implants very fast, creating a biofilm which is difficult for the host immune system to remove even in conjunction with antibiotics [7]. The more attractive the surface of an implant is for healthy body cells, the smaller the chance that a biofilm can develop. Thus there is a pressing need to develop a more refined interface which better manages the FBR while facilitating normal wound healing and prevents biofilm formation. These requirements may be fulfilled by employing hydrogels as an interface material.

Hydrogels are biocompatible, three-dimensional porous structures capable of swelling in water or biological fluids while retaining large amounts of water/fluid in the swollen state giving hydrogels a rubbery appearance with mechanical behavior similar to that of natural living tissue. Furthermore, hydrogels are non-attractive surfaces for proteins and cells to adhere due to its low interfacial free energy in contact with body fluid. On the other hand hydrogels can be modified, allowing the immobilization of proteins, cells and other molecules [8, 9]. These and other properties of hydrogels resulted in many applications ranging from food additives and pharmaceutical applications to tissue engineering. Hydrogel scaffolds have also been employed for the improvement of the biocompatibility of implantable biosensors [10,11]. For a good performance of a hydrogel after implantation, it must possess suitable porosity to allow penetration and proliferation of cells. In addition the hydrogel must also have suitable mechanical properties [12-14].

In this work we report a method that is, to our knowledge, a unique way to modify the pore size and improve the elastic properties of 2-hydroxyethyl methacrylate (HEMA) based hydrogel. As a result, hydrogels were obtained which exhibit penetration and proliferation of fibroblast cells, hence improved body reactions upon implantation might be expected. Moreover, different porosity characterization techniques for hydrogels were investigated and compared.

II. Materials and Methods

Materials: 2-hydroxyethyl methacrylate (HEMA), 1-vinyl-2-pyrrolidon (VP), ethylene glycol dimethacrylate (EGDMA), N-N'-methylenebis (acrylamide), alginic acid, calcium chloride and 1,1'-carbonyl diimidazole (CDI) were supplied by Sigma-Aldrich. 2,3-dihydroxypropyl methacrylate (DHPMA) was supplied from Gelest. Gelatin (mesh size 30) was donated from Rousselot. Type I rat tail collagen was supplied by Gibco. All materials were used as received.

Methods: Four different hydrogel compositions were prepared, referred to as A1, A2, A3 and A4. For preparing A1, a solution of HEMA, DHPMA, VP, EGDMA and N-N'-methylenebis (acrylamide) (molar ratio 3:4:3:1:1) was prepared. The solution was further diluted in water. A1 was the base line sample for the experimental work.

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A2 was prepared following a similar procedure to A1 but a 50% (by volume) alginate solution was added. Alginate was used to modify the mechanical properties of the hydrogel.

A3 and A4 were prepared in the same way than A1 and A2 respectively but 100mg gelatin was added per 1ml of mixture. Gelatin was used as a porogen to influence the porosity.

All solutions were placed in a mold and crosslinked by UV irradiation (302 nm) for 120 minutes. After crosslinking, samples containing alginate were treated with an ionic solution to crosslink the alginate, and samples containing gelatin were soaked in distilled water at 90º C to remove the gelatin used for pore generation. All samples were stored for one week in distilled water to remove any unreacted monomers, the water was refreshed daily.

Collagen type I was immobilized into the hydrogels following the procedure described in Bryant *et al.* [15]

III. Hydrogel Characterization

A. Porosity Characterization Methods

Initially porosity characterization is carried out by scanning electron microscopy (SEM) since it is a highly used imaging technique for hydrogels. Prior to SEM inspection, samples are dried by lyophilization and sputtered with gold. While using this technique for evaluating porosity of hydrogels, non-reproducible results were observed, hence the characterization technique itself was investigated, revealing issues with the lyophilization process. In order to study its reproducibility, a hydrogel sample is sectioned in four pieces and dried via lyophilization on different days under the same conditions (Fig.3).

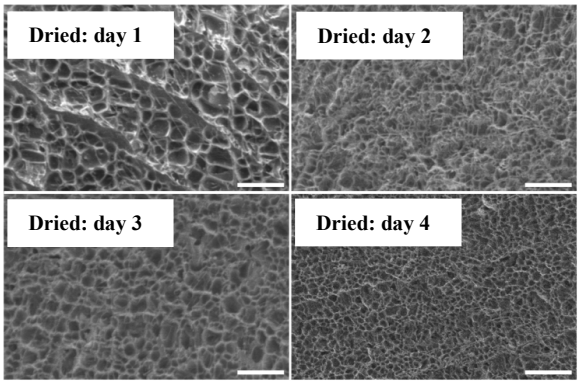

Fig.3: Four SEM images from same sample (A1) dried by lyophilization on 4 different days showing a poor reproducibility. The scale bar corresponds to 20μm in all images.

From Fig. 3 various pore sizes and morphologies are present which suggests that the lyophilization technique for porosity characterization is not the best option since an obvious poor reproducibility is obtained. As an alternative, the use of an environmental scanning microscopy (ESEM) for porosity characterization is proposed.

ESEM inspection is performed on samples dipped in liquid nitrogen immediately before ESEM evaluation. The ESEM is equiped with a cooling stage to keep the sample frozen during the imaging process. Very clear ESEM images are shown in Fig.4. The results strongly suggest that the ESEM characterization is more representative of the actual material. In addition, characterization of the hydrogel pore structure by ESEM has the advantage that sample preperation is less time consuming.

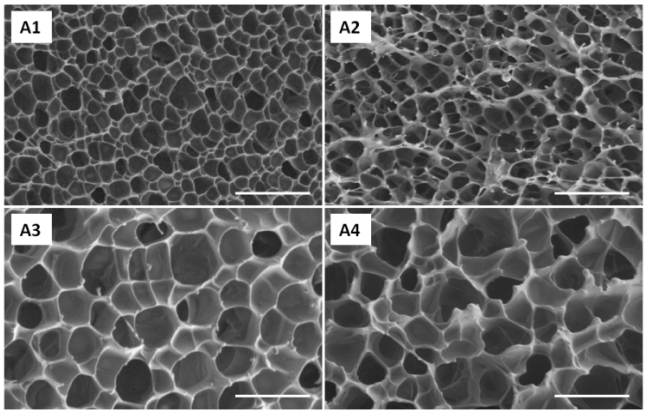

Fig.4 ESEM images of frozen hydrogels samples. Scale bar corresponds to 10μm in all images.

B. Porosity Evaluation

In Fig. 4, samples A3 and A4 show the larger interconnected pore network which results from the addition of gelatin during the synthesis of the material. It is believed that phase seperation of the gelatin and the hydrogel components in the initial mixing of the hydrogel components is responsible for the formation of the pore network. This gives us the ability in the future to tune the pore size of the material by selecting gelatin of diferent mesh sizes.

Non-alginate containing hydrogels (A1 and A3) exhibit very uniform porosity while the alginate containing samples (A2 and A4) show more variety in pore size..

C. Swelling behavior

Water sorption tests are conducted to monitor the uptake of water by the hydrogel. The hydrogel samples are dehydrated and the weight is recorded. The samples are then immersed in water and their weight is recorded at different time intervals. Care is taken to remove any excess of water before measuring. Monitoring continued until the samples reached a constant mass value. The sorption results are shown in Fig. 3.

The sorption degree (SD) is calculated following the equation:

$$SD\% = \frac{M_t - M_d}{M_d} \times 100 \qquad (1)$$

where M_t is the mass at different intervals of time and M_d is the mass of the dry hydrogel.

Samples A2 and A4 containing alginate exhibit a higher water sorption due to the presence of additional hydroxyl groups in the alginic acid which increases the hydrophilic nature of the hydrogel. Samples A1 and A3 have less water

sorption due to the shortage of these hydroxyl groups. The water content of the hydrogels plays an important role in the mechanical properties of the material, explained below in greater detail.

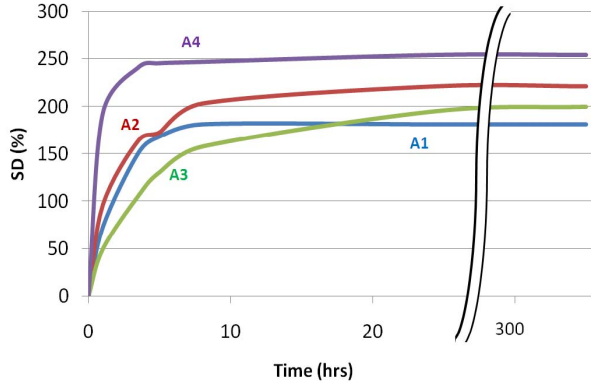

Fig.3: Swelling degree as a function of time for different samples.

D. *Mechanical Testing*

Hydrogel samples with a cylindrical shape are prepared for mechanical testing. The mechanical behavior of the hydrogels is measured using a load cell. A special sample holder is adapted to perform the compression test in water and to prevent movement of the sample or hinder the expulsion of water from the hydrogel during testing. The measurements are performed at a speed of 10µm/sec resulting in a force-displacement curve. A stress-strain curve is calculated with the following equations:

$$Stress\ (Pa) = \frac{L}{A} \tag{2}$$

where L is the load measured in N and A is the cross-sectional area of the sample measured in m^2.

$$Strain\ (\%) = \frac{d_0 - d}{d} \times 100 \tag{3}$$

where d_0 is the initial displacement position and d is the total displacement position. The obtained stress-strain curves are shown in Fig. 4 representing the elastic behavior of the different hydrogel compositions.

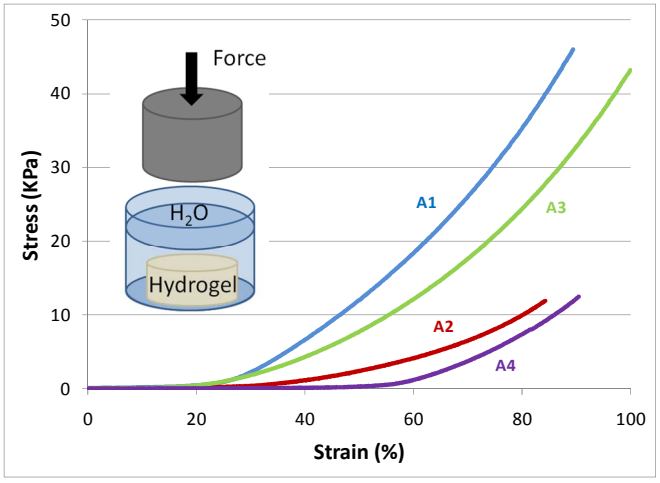

Fig.4: Stress-strain curve obtained from compression tests on hydrogels.

Samples A2 and A4 containing alginate, exhibit a softer behavior due to the higher water content. With increasing porosity size (compare A1 with A3 and A2 with A4) also the elasticity of the material is enhanced. During the manual handling of the material, the alginate containing samples also displayed a more durable (less brittle) behavior than the samples without alginate. Elasticity and durability are important properties, enabling easy manipulation of the material before and during implantation, and minimizing the undesirable effect of flaking or cracking of the coating material even after implantation.

E. *Affinity of hydrogels for cell proliferation*

As mentioned in the introduction, cells have a low affinity for hydrogels. To improve cell migration into the hydrogel and further proliferation, collagen type I is immobilized into the hydrogels as previously described in section II.

For *in-vitro* testing, the fibroblast 3T3 cell line is used. Cells are incorporated in each sample and centrifuged to encourage the infiltration of cells into the hydrogels. After 4 days of culturing, the hydrogel samples are fixed with a fixation buffer and rinsed in PBS. Cells are studied by fluorescence microscopy. Propidium iodide and Hoechst dye is used for staining the cell to evaluate the presence of cells and to study the cell morphology.

To determine the influence of collagen in the hydrogel, cells are cultured in various hydrogel samples with and without collagen. Fluorescence microscopy images of one sample (A4) with and without collagen are showed in Fig.5.

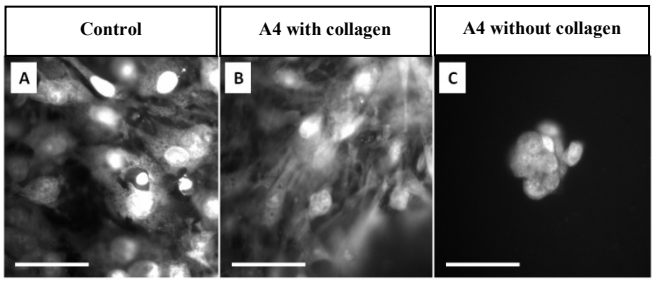

Fig.5 Images from fluorescence microscopy: (a) control sample, (b) sample A4 with collagen immobilized and (c) A4 sample without collagen. Scale bar corresponds to 50µm in all images.

Fig. 5A is a positive control showing a healthy population of cells. Sample A4 with collagen immobilized (Fig.5B) exhibits a large healthy population of fibroblast very similar to the control. On the other hand, in the sample without collagen (Fig. 5C) it was difficult to locate viable cells populations, however a few clusters of cells are found but their appearance shows that they are non-viable cells.

Fibroblast 3T3 cells are not visible in samples processed without gelatin having smaller pores (Fig.6, A1 and A2). However, viable cells in vast numbers are seen in the cell culture dish of sample A1 and A2, but not on the samples, only around these samples. This shows that the hydrogel samples are non-toxic but not adequate as a cell scaffold-like

material. It is known that fibroblasts require adherence to a suitable surface otherwise they will not proliferate.

On the other hand, a healthy cell population is visible on samples with larger pores (Fig.6, A3 and A4). The sample combining larger porosity and higher elasticity (Fig.6, A4) is showing the larger cell population.

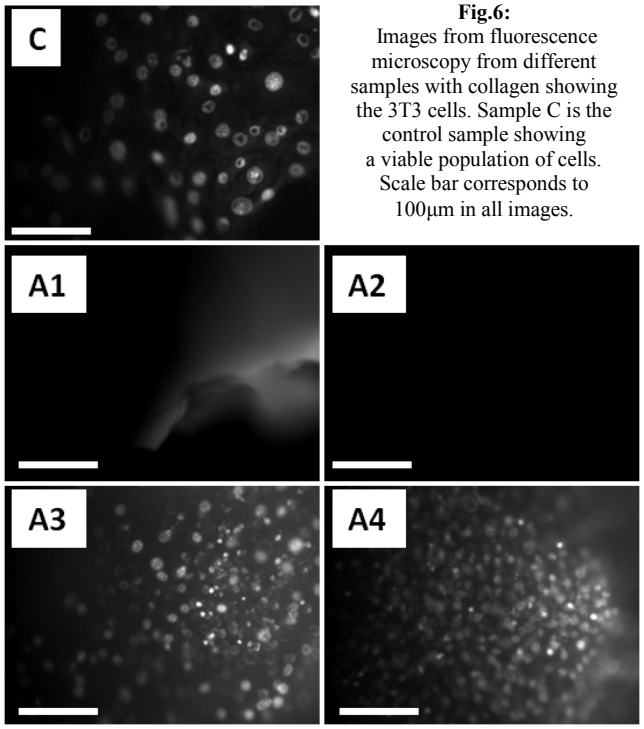

Fig.6: Images from fluorescence microscopy from different samples with collagen showing the 3T3 cells. Sample C is the control sample showing a viable population of cells. Scale bar corresponds to 100μm in all images.

IV. Conclusions

Biocompatibility of hydrogels strongly depends on the mechanical nature of the material and the presence of guest biomolecules within the material matrix. We have successfully shown that using a combination of different hydrogel components, which in the past have been used independently, we can design a hydrogel with properties attractive to fibroblast cells.

We discovered inadequacies with existing methods to characterize the pore structure of hydrogels and overcame these shortcomings with a more reliable characterization technique based on ESEM.

Utilizing gelatin as a porogen during synthesis, a hydrogel with greater pore sizes is achieved, which makes the hydrogel more attractive to fibroblast cells. While not being cytotoxic, hydrogels with the smaller pore sizes proved being not attractive for fibroblasts and hence no viable cell populations are observed on the surface of such hydrogels.

A difference was also seen when alginate was incorporated into the hydrogel synthesis. The alginate containing hydrogels had improved mechanical properties, and some variety in pore size is observed.

Finally the immobilization of collagen as a guest biomolecule is performed succesfully, resulting in viable cell populations for those hydrogel materials which exhibit larger pore sizes.

These incremental advancements in hydrogel design will, in the future lead to a material with the correct requirements for use as an interface/packing material for implantable biomedical systems.

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