PAN AFRICAN SCHOOL OF MATERIALS (PASMAT) 2015

MATERIALS FOR BIOMATERIALS WORKSHOP

Polymer-based Implantable Biomedical Device Fabrications

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Instructor(s): Dr. Danyuo Yiporo, Dr. Olushola S. Odusanya and Mr. Ani J. Emeka.

1.0 Introduction

The increasing incidence of cancer [1] has stimulated research on the development of novel implantable devices for the localized treatment of cancer [2-4]. Cancer is currently the second leading cause of death worldwide after cardiovascular disease [5, 6]. Current trends also suggest that cancer will become the leading cause of death by 2030 **[**5, 7**].** Furthermore, standard treatment methods, such as bulk systemic chemotherapy [1, 4, 8] and radiotherapy [9-11], have shown severe side effects. There is, therefore, the need to develop localized cancer treatment methods to mitigate these side effects.

One approach that can be used to reduce the potential side effects of cancer treatments is to use localized drug delivery that can reduce the higher concentrations of cancer drugs in a tissue. This can be achieved by using implantable drug eluting devices for the localized delivery of drugs [4, 12]. Such approaches can also be combined with localized hyperthermia in cancer treatment [13, 14]. Recent research by Yaoming *et al.,* (2012) [12], has also shown that haematoporphyrin based-photodynamic therapy, combined with hyperthermia, provides an effective therapeutic vaccine against colon cancer growth in mice.

The uptake, storage and delivery of cancer drugs can be facilitated by the use of gels [16-18]. These include environment-sensitive gels that can respond to local stimuli, such as temperature, pH, electric fields and solvent composition [2, 19-21]. The swelling and controlled release of cancer drugs [22, 23] from such gels can, therefore, provide the basis for the design of implantable biomedical systems for the localized treatment of cancer. However, such controlled release requires a good basic understanding of phase transitions [22, 23], swelling and diffusion-controlled release from smart hydrogels.

Thermo-sensitive hydrogels have been explored for their potential use in drug delivery [4, 12, 24, 25]**.** These include poly(N-Isopropyl acrylamide) P(NIPA), which is a thermo-sensitive hydrogel. PNIPA has a lower critical solution temperature (LCST) of about 32˚C in aqueous solution, especially when it has been cross-linked [12, 26]. P(NIPA) is produced by reacting TEMED with P(NIPA)-based gels through free radical polymerization. The process is terminated by exposing the samples to air. Freezing the samples below 9˚C also helps to produce heterogeneous microporous hydrogels with interconnected pores. It has also been reported that the LCST of P(NIPA) is dependent on the pH, with the LCST increasing with increasing pH [27].

Furthermore, crosslinking P(NIPA) with acrylamide helps to effectively increase the LCST, while cross-linking with butyl-methylacrylate decreases the LCST [4]. Such control of the LCST makes P(NIPA)-based gels attractive for potential applications in drug delivery systems. However, there is a need for further research to prepare NIPA-based gels for potential applications in drug delivery systems for the localized treatment of diseases such as cancer.

Projects:

1.1 Poly (Di-methyl-Siloxane) (PDMS) Fabrication

1. Obtain aluminium and bronze molds from the instructor(s) with screws.
2. The molds contains holes with a diameter of 1.12 mm drilled into four locations
3. PDMS packages with different channel lengths and reservoirs are obtain by mixing sylgard 184 kit silicon elastomer with a silicon elastomer curing agent (a cross linker). These should

be mix in a ratio of 10:1 v/v and 50:1 v/v ratios.

4. Stir the mixture vigorously, de-gas with a GALVAC vacuum oven set at -24 mm Hg equivalent, with no heat, for about an hour.
5. Fix the molds with the aid of nuts and bolts given to you, while 1.12 mm diameter thick surgical needles are pass through the four faces to produce the micro-channels.
6. In order to induce temperature responsiveness in the gels, 5-10 turns of thin copper wire can be incorporated into some devices to induce Joule heating.
7. Pour the de-gassed PDMS gently into the fabricated molds.
8. The samples can then be cure at 60˚C for 3 hours or at room-temperature (28˚C) for 12 to 24 hours.

1.2 Preparation of P(NIPA)-Based Hydrogels

1. P(NIPA)-based hydrogels are usually prepared by free radical polymerization. The concentrations of Amonium persulphate (APS) and methylene bisacrylamide (MBA) should about 1.91 mol% and 1.15 mol%, respectively.
2. The amount of the cross-linker (MBA) was obtained from:

$$\frac{\left(\frac{M_{MBA}}{M_{w(MBA)}}\right)}{\left(\frac{M_{NIPA}}{M_{w(NIPA)}}\right)} x\ 100\% = mol\ \% \qquad (1)$$

   where, $M_{w(NIPA)}$ is the molecular weight of the monomer, NIPA $\left(113\ \frac{g}{mol}\right)$, M_{NIPA} is the initial amount of the monomer (0.87 g), $M_{w(MBA)}$ is the molecular weight of the cross-linker, MBA $\left(154\ \frac{g}{mol}\right)$ and M_{MBA} is the unknown amount of MBA (g) to be used in the gel polymerization. The mole% of MBA was 1.15 %. The calculated amount of the MBA based on the 0.87 g of NIPA (monomer) from equation (1) should be 0.0136 g.
3. The amount of APS should be obtained by replacing MBA in equation (1) with APS.
4. P(NIPA)-based homo-polymer, denoted by gel code A, are prepared by mixing 0.87 g of NIPA, 0.0136 g of MBA, and 0.0335 g of APS (summarized in table 1).
5. The samples can then be dissolve with 7.8 ml of DW, before stirring vigorously until a homogenous mixture is obtain from 4-10˚C.
6. The mixing process may be exothermic. The solution should then be immerse in ice, and degas with a vacuum oven for 20 minutes.
7. The homo-polymer can be initiated with 15 µl of TEMED. The mixtures should be swirl gently for 5-10 seconds.
8. Subsequently pour the solution into 5 mm diameter cylindrical molds, well open to terminate the free radical polymerization.
9. The samples inside the cylindrical molds could be left at 24˚C in a water bath to strengthen the polymerized gels over 6-12 hr period.
10. Samples should be wash for about 10 times with deionized water to remove any chemical residue.
11. The resultant wet gels can be cut into discs and cylindrical samples with diameters of 5 mm and heights of 5 mm.

12. Soak the samples in deionized water, while replacing the deionize water at regular time interval.
13. Remove the samples from the deionize water and subsequently dry in a laboratory environment (29˚C)/in a vacuum at 40˚C to remove all moistures.
14. P(NIPA)-based co-polymer hydrogels can also be prepare using the same procedure for the fabrication of the P(NIPA) homo-polymer (see Table 1 for details).
15. Co-monomer species, AM and BMA can be co-polymerize with P(NIPA) to form co-polymers gels.
16. Samples of P(NIPA) co-polymer hydrogels with 5 or 10 mol% of BMA should be initiated with 5 and 10 µl of TEMED, respectively, while gels that contained 5, 10 and 15 mol% of AM can also be initiated with 20, 30, 40 µl of TEMED, respectively. Control additions of TEMED will reduced the turbidity of the hydrogels to produce transparent hydrogels.

Table 1: P(NIPA)-Based Hydrogel Configuration and their Compositions

Gel Code	P(NIPA) (mol%)	AM (mol%)	APS (g)	MBA (mg)	TEMED (µl)	BMA (mol%)
A	100	-	0.0335	0.0136	5	-
B	95	5	0.0335	0.0136	10	-
C	90	10	0.0335	0.0136	20	-
D	85	15	0.0335	0.0136	30	-
E	95	-	0.0335	0.0136	5	5
F	90	-	0.0335	0.0136	10	10
G	90	5	0.0335	0.0136	20	5
H	85	5	0.0335	0.0136	30	10

1.3 Characterization and Swelling/Drug Release Kinetics

1. The transport characteristics of P(NIPA)-based hydrogels are largely dependent on the morphologies of the gels inner matrices. The optical images/videos of the dry gels can be determine with LCD Deluxe digital optical microscope or proscope imaging system.
2. Determine also, the average porosity or pore ranges of the gels obtain by your group using proscope image analyzer and compare your result with others.
3. Study the swelling (SR_1) and re-swelling (SR_2) kinetics from the hydrogels from the equations below and compare your results.

$$SR_1 = (M_t - M_o)/M_o \quad (2a)$$
$$SR_2 = (M_t - M_o)/M_o \quad (2b)$$

where M_t is the mass of the gel at time t and M_o is the mass of the dried gel at time, t = 0.

4. Soak gels in fluid/drug solutions to saturate and study the drug release kinetics.
5. The fluid release exponent, n, and the diffusion constant, k, should be determine from the power law equation:

$$\frac{m_t}{m_o} = 4\left(\frac{Dt}{\pi\delta^2}\right)^n = kt^n \quad (3)$$

where $\frac{m_t}{m_o}$ is the fluid or drug release fraction at time, t, δ is the thickness of the gel and D is the diffusivity.

6. The constants k and n can be obtain from the linear form of equation (3). This gives:

$$ln\left({}^{m_t}/_{m_o}\right) = \log k + n \log t \quad (4)$$

where k and n are obtain, respectively, from the intercepts and slopes of the plot of $\ln(m_t/m_o)$ versus ln t (s).

7. The diffusion coefficient, D, can be obtain from:

$$D = \frac{k\pi\delta^2}{4} \quad (5)$$

8. If you can afford to do the experiment for different temperatures, the activation energy for the gels can be obtain from the Arrhenius equation:

$$D = D_o exp\left(\frac{-E_a}{RT}\right) \quad (6)$$

where D is the diffusivity, D_o is the diffusion constant, R is the universal gas constant, T is temperature, E_a is the activation energy for each gel.

9. Discuss the implications of your results?

1.4 Encapsulation of P(NIPA) into PDMS Capsules

1. PDMS capsules consist of sylgard 184 kit silicon elastomer and a silicon elastomer curing agent of 10:1 and 50:1 ratio by volume as presented in section 1.1.
2. Drug loaded PNIPA-based hydrogels can then be insert into the reservoir of the PDMS capsules.
3. Apply a thin layer according to point (1). The two layers can be properly seal using a clamping device to apply a slight pressure to the axes perpendicular to the edges.
4. Sealed packages can then be incubated at 40˚C for 24 hours to ensure that the two layers stuck together tightly.
5. Subject the devices to joule heating at (37-45˚C) to simulate potential exposures to normal body temperature (37˚C) and hyperthermic temperature ranges (43-45˚C).
6. Determine the time require for the fluid to flow across the devices with different channel lengths. The effective diffusion coefficient, D is given by:

   $L = \sqrt{Dt}$ *(1)*

   where L is the channel length and t is the time taken for the fluid to flow across the channel length.

7. The effective diffusion coefficient, D, across a different channel length, L, can be obtained from D = L^2/t, where t is the duration of flow. Obtain a plot of L^2 versus t.

1.5 Fluid Release Rate

The drug/fluid release from the loaded gels (at temperatures between 28˚ and 48˚C), can be obtain using the early-time approximation. This model assumes Fickian diffusion. The release rate is then dependent on $t^{-0.5}$. This gives:

$$\frac{dM_t}{dt} = 2M_{total}\left(\frac{D}{\pi\delta^2 t}\right)^{1/2} \tag{7}$$

where the above constants have their usual meaning. The release rate is best described by the form:

$$\frac{d\left(M_t/M_{total}\right)}{dt} = 2\left(\frac{D}{\pi\delta^2 t}\right)^{1/2} \tag{8}$$

Obtain a plot of $\frac{d\left(M_t/M_{total}\right)}{dt}$ versus $t^{-0.5}$.

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