CL 409: Material Science Project

Department of Chemical Engineering

Biosensors for Prostate Cancer

Team Material Madness

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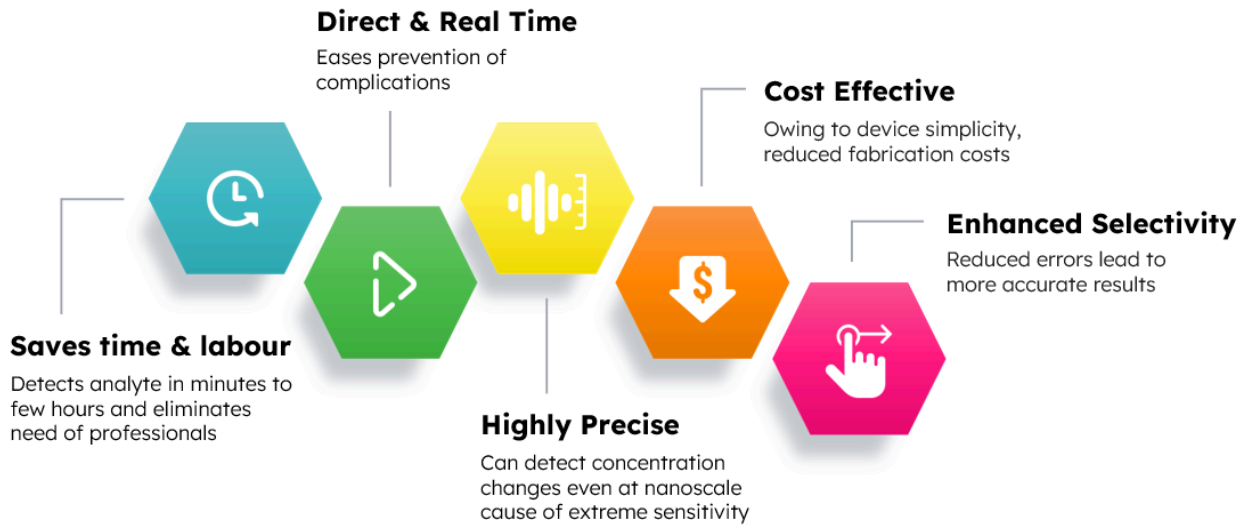
INTRODUCTION

What are Biosensors?

A biosensor is an analytical device that detects chemical or biological reactions using biological components, such as an enzyme, antibody, nucleic acid hormone, organelle, or whole cell. Biosensors are utilized in various applications, such as medical diagnostics, environmental monitoring, process monitoring, the food industry, etc.

Its 2 major components are a bioreceptor and a transducer. The receptor behaves like a sensor and reacts with the analyte to generate an electrical, optical, or thermal signal. The transducer, which can be a semi-conducting material or nanomaterial. Transducer amplifies the biochemical signal received from the bio-receptor and alters the resulting signal into electrical signals that can be measured. The electrical signals are then displayed in an attainable way using an electrical circuit consisting of a signal conditioning unit, a processor or micro-controller, and a display unit.

Why Biosensors?



PROPOSED DESIGN

Overall Design

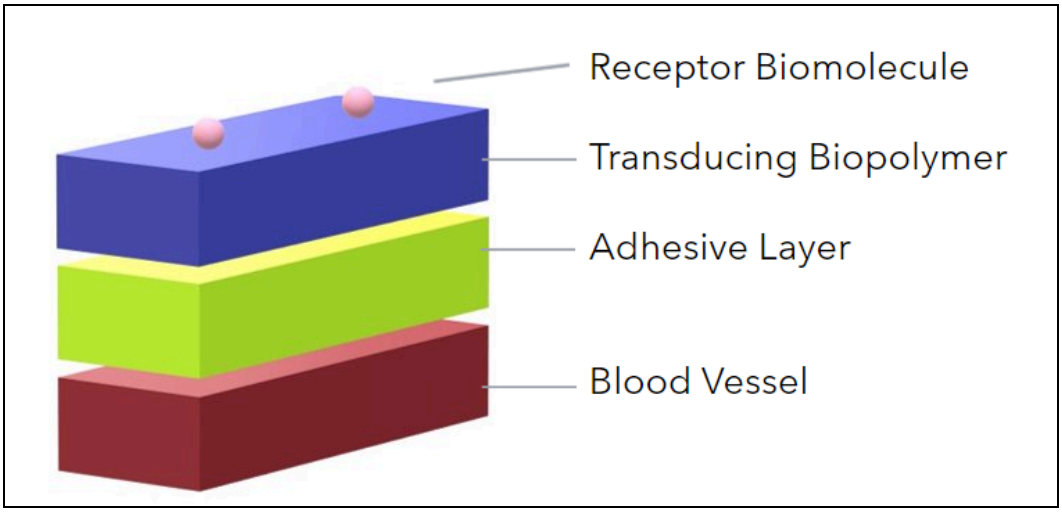


Figure 1: Layers of a biosensor in vivo

1. Choice of Adhesive

The hydrogel, called alginate-catechol, is formed by coupling alginate, a biostable polysaccharide, with dopamine hydrochloride to create adhesive properties. The adhesive strength of the gel arises from catechol moieties within the structure, mimicking the adhesive chemistry of marine mussels. The gel covalently cross-links when oxidized using sodium periodate, resulting in strong adhesion.

Structure

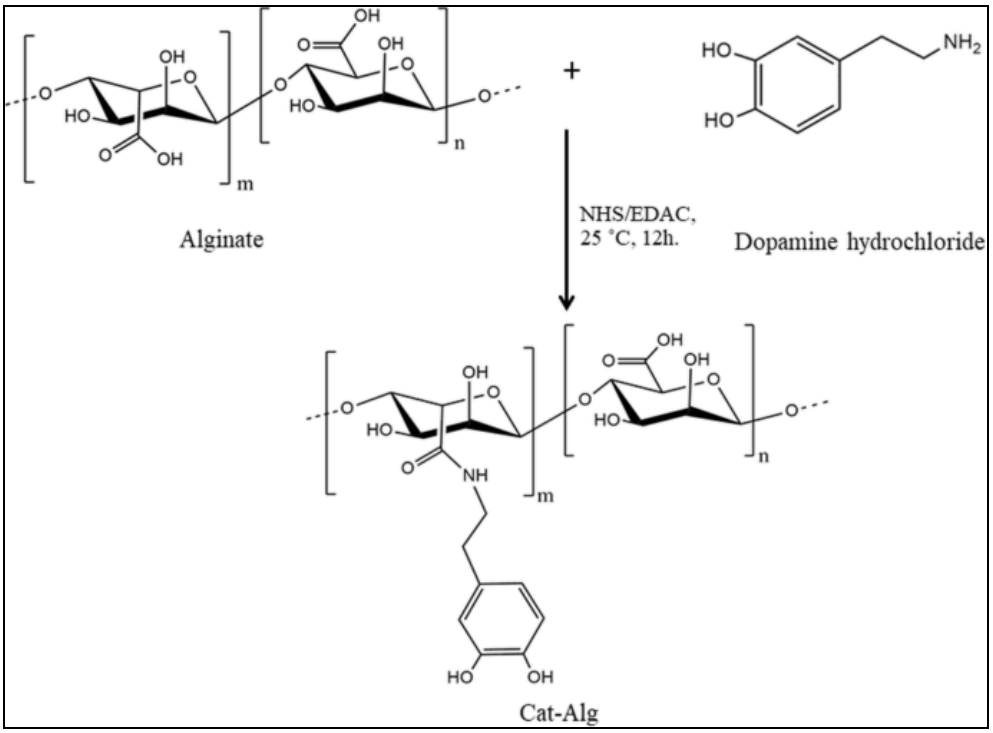


Figure 2: Chemical Structure of Alginate-Catechol

Mechanism of adhesion

The mechanism by which catechols adhere to cellular surfaces is a combination of hydrogen bonding through phenolic protons and covalent bonding through the reaction of nucleophiles on the surface of cells and matrices with the unstable quinone group formed by oxidation of catechols.

The gel covalently cross-linked in 10–20 min following the addition of periodate, an oxidant that has been used to cross-link catechol-containing hydrogels in vivo for long-term implantation of islets

Degradation time

The gel was still present in vessels 4 months after treatment in the two mice that were tested then.

Will it stick on the polymer as well as blood vessels?

Yes, the adhesive gel you synthesized can bond to another polymer. Alginate is a polysaccharide that can form a biostable and generally biocompatible hydrogel, while catechol provides sites for cross-linking and adhesive properties. This means the adhesive gel can form strong bonds with biological and synthetic materials.

The most likely type of bond that will form with the polymer is a hydrogen bond. Alginate and catechol both have functional groups that can form hydrogen bonds, such as hydroxyl groups and amino groups.

Physical Properties like Tensile Strength

The adhesive shear strength of the gel was 12 Pa, a shear stress several times higher than that generated by physiological blood flow. In a lap-shear tensile strain test, the gel was applied to the inside of a bovine carotid artery that had been excised for 3 d and had denuded endothelium. The shear strength of the gel to the artery was 2 ± 1.3 kPa, which is two to three orders of magnitude higher than physiological shear stress.

Other places where it was used: The treatment of diseased vasculature.

Mechanism of putting it in the body

The carotid artery was temporarily ligated to stop blood flow, a catheter containing a solution of alginate-catechol and fluorescent particles was inserted through an incision in the artery and positioned away from the incision, and 0.3–0.6 μL of the solution was applied through the catheter to place the viscous solution in contact with the vessel wall. In our case, we can use the Aorta artery.

2. Choice of Polymer

The polymer should satisfy the following needs:

a. Attaches well with Biomolecule (Covalent bonds needed)
b. Attaches well with adhesive (Polar groups needed)
c. Resistance to Tear (Tensile Strength > 2 Pa[1])
d. Flexible (Low modulus of Elasticity)
e. Biocompatible with blood
f. Electrically conduction
g. Cheap

The material of choice is 15-30% of Multi-Wall Carbon Nanotube with a biocompatible polymer[2]. Based on the above criteria, we can reject UHMWPE, PMMA and PP from the biopolymer list.

Property	PTFE	Silicones	PDMS	PET
Biocompatibility	Good	Good	Excellent	Good
Cost	Rs 2300/Kg	Rs 350/kg	Rs 250/Kg	Rs 122/kg
E	0.5 Gpa	0.01 Gpa	0.002 Gpa	3.5 GPa
Tensile Strength	0.02 GPa	0.003 GPa	0.004 GPa	0.05 GPa
Affinity to Water	Hydrophobic	Hydrophobic	Hydrophobic	Hydrophobic
Contact angle with Water	106.94°	112°	107°	75°

Note that PDMS is the most optimal polymer except the Hydrophobicity. To solve this, we use a copolymer of PEO and PDMS[3]. PEO brings hydrophilicity and attachment to adhesive while the PDMS brings hydrophobicity and attraction to the biomolecule. Moreover, PDMS is flexible (E = 0.002 GPa) while PEO is not (E = 0.25 GPa). This is a classic example of an amphipathic polymer.

[1] https://doi.org/10.1073/pnas.1217972110
[2] Callister, W. D., & Rethwisch, D. G. (2009). Materials Science and Engineering: An Introduction (8th ed.).
[3] https://doi.org/10.1021/ma0514828

3. Biomolecule Characteristics

a. **Specificity and Selectivity:** The bio-receptor should be highly selective for the target analyte, ensuring that it does not interact with other closely related compounds. This specificity is crucial for accurate and reliable detection.
b. **Sensitivity:** The bio-receptor should exhibit high sensitivity to detect even low concentrations of the target analyte. This is important for applications where trace amounts of the analyte need to be measured.
c. **Stability:** The bio-receptor should maintain its activity and specificity over time and under various environmental conditions. Stability is crucial for the long-term performance and reliability of the biosensor. It should also have high reproducibility.
d. **Affinity:** The bio-receptor should have a high affinity for the target analyte, ensuring strong and specific binding interactions. This is often measured by the dissociation constant (K_d), which represents the strength of the binding between the bio-receptor and analyte.
e. **Ease of immobilization:** The bio-receptor should be easily immobilized onto the sensor surface without losing its activity. Immobilization methods should maintain the bio-receptor's orientation and functionality.

Immobilization is most commonly achieved through the following bonding types:

1. **COVALENT BONDING**

   Direct covalent binding is the most widely used enzyme immobilization technique, in which the biorecognition element is firmly bonded either to the electrode/transducer surface or to the inert matrix of the membrane. The binding mechanism depends on the interaction between biorecognition elements and functional protein groups (usually side chains of amino acids) and reactive groups of the transducer/membrane matrix surface. The advantages of direct covalent binding include strong resistance to environmental changes, little leakage of biorecognition element (enzyme), and strong bond formation between the biorecognition element (enzyme) and matrix. The main disadvantage of this method is that the developed matrix cannot be regenerated once used.

2. **CROSS LINKING**

   The mechanism occurs via the creation of intermolecular cross-linkages between biorecognition elements (enzymes) or between biorecognition elements and functionally inert proteins (for example bovine serum albumin). This process is

performed with the help of multi-functional reagents that act as a linker to connect enzyme molecules in 3D cross-linked aggregates to the transducer surface. It allows shorter response time, stronger attachment, and higher catalytic activity of enzymes. The advantages are less leakage of enzymes, stronger chemical binding, and the possibility to adjust the environment for the biorecognition element using appropriate stabilizing agents, The disadvantages are the formation of covalent cross-links between protein molecules instead of the matrix and protein, and that partial denaturation of protein structure limit the application of cross-linking immobilization.

Advantages that our model provides

- Strong attachment to body (via adhesive layer) increases stability
- We pick *covalent bond* immobilization between transducer-sensor, which ensures high signal strength
- Distributed sensor molecules counter local eddies for accurate and reliable signals
- Use of MWCNT makes the device more sensitive
- Use of this assembly increases reproducibility, stability and selectivity

CASE STUDY

Choice of Disease

To make a proof-of-concept, Prostate Cancer is chosen as the target disease. The prostate is a small walnut-shaped gland in males that produces the seminal fluid that nourishes and transports sperm.

Unfortunately, prostate cancer is one of the most common types of cancer. Many prostate cancers grow slowly and are confined to the prostate gland, where they may not cause serious harm. However, while some types of prostate cancer grow slowly and may need minimal or even no treatment, other types are aggressive and can spread quickly.

Prostate cancer that's detected early — when it's still confined to the prostate gland — has the best chance for successful treatment.

Prostate cancer has strong genetic factors hence, a lot of people already know that they are at high risk. Additionally, the fact that 2-3% of men die from this condition and 12.5% of men are diagnosed with it highlights the severity of this illness. To be sure a man doesn't have it, the examination should be performed every six months. However, because this occurs far too frequently for the busy people of today, using a biosensor and having a continuous real-time assessment of the condition is easier.
Prostate-specific antigen testing and prostate biopsies, two labor-intensive conventional diagnostic procedures, are also available. Therefore,other strategies must be employed.

Choice of Biomolecule

After selection of disease we must select what metabolite activity pertaining to prostate cancer should be recorded and how can it be executed via a suitable biomolecule? Various markers showing significant changes in this particular condition (prostate cancer in this case) were studied. These were Citrate[4,5], Tyrosine[6], 3D-hydroxybutyrate[7], Spermine[8] and Myo-inositol[9].

After rigorous comparative analysis upon the basis of the biomolecule it attaches to, 3D-hydroxybutyrate was finalized. 3DHB is highly upregulated in prostate cancer, hence is a very potential marker for keeping check on the disease. The receptor molecule which senses the metabolite, in this case 3-hydroxybutyrate dehydrogenase (3HBDH), an enzyme acts upon the compound and oxidizes it to acetoacetate.

MAIN REACTION

(R)-3-hydroxybutyrate + NAD^+ ⇌ Acetoacetate + NADH + H^+

Immobilization of 3HBDH

For the enzyme to present there for a great interval of time and to keep it in a stable environment, protecting from denaturation and leaching, therefore it needs to be immobilized to the transducer polymer. 3HBDH has the property of linking covalently to Single/Multi walled CNTs. Since the transducing polymer, PEO-PDMS has been doped with 15-30% MWCNTs , immobilization is achieved successfully (one more reason why CNT composites are used).

CNTs are covalently linked to NAD^+, the cofactor for the enzyme reaction. NAD^+ is constantly regenerated by oxidation of NADH which is present as the product of the reaction; it is a cyclic process.

[4] https://doi.org/10.1371/journal.pone.0028245
[5] https://doi.org/10.1021/acscentsci.0c00518
[6] https://doi.org/10.1039/D0RA05581F
[7] https://doi.org/10.1109/JSEN.2022.3229474
[8] https://doi.org/10.1007/s00216-014-8324-4
[9] https://doi.org/10.1155/2022/3998338

Measurement of current proportional to NADH concentration[10]

The biosensor measures the current of the oxidation peak of NADH produced in the given reaction. The key is the extraction of electrons from NADH to the electrodes and converting them into an electric current. This current is again measured with the help of CNTs as they are extremely good for conduction of electrons.

[10] https://doi.org/10.1109/JSEN.2022.3229474

MARKET ANALYSIS

Conventional Tests

A rapid market scan is undertaken to assess the pricing of diverse screening and diagnostic tests within the market for the detection of prostate cancer.

1. Prostate-specific antigen (PSA) blood test : The amount of the PSA in the blood is measured and different grades are assigned on the basis of the severity of the cancer. A PSA test is used to screen for prostate cancer. Cancer screening means looking for signs of cancer before it causes symptoms. The average market price is in the range ₹800-₹1000.
2. Digital rectal exam : For a digital rectal exam (DRE), the doctor inserts a gloved, lubricated finger into the rectum to feel for any bumps or hard areas on the prostate that might be cancer. Prostate cancers often begin in the back part of the gland, and can sometimes be felt during a rectal exam.
3. Prostate biopsy : During the biopsy, the doctor usually looks at the prostate with an imaging test and quickly inserts a thin, hollow needle into the prostate. When the needle is pulled out it removes a small cylinder (core) of prostate tissue. This is repeated several times. The average market price is in the range ₹4000-₹10000.
4. Imaging Tests: If your doctor suspects your cancer may have spread beyond your prostate, one or more of the following imaging tests may be recommended:

- Transrectal ultrasound (TRUS) : The average market price is in the range ₹1000-₹2000
- Magnetic resonance imaging (MRI) : The average market price is in the range ₹4000-₹9000
- Positron emission tomography (PET) scan : The average market price is in the range ₹12000-₹25000
- Bone Scan : The average market price is in the range ₹3000-₹10000.
- Computed tomography (CT) scan : The average market price is in the range ₹10000-₹35000

Our Biosensor

We are analyzing the cost of raw materials and taking that as the ballpark cost for the assembly.

1. Polymer

References of the calculations were taken from this paper[11]. Important figures are reproduced here for reference:

Component	Cost ($/kg)	Formulation(%wt)
MWCNT	100	8-10
Carbon black	10	0-8
Polymer	1	71
Bismaleimide	10	10
Furfurylamine	10	10

Formulation	Cost of Components ($)					Cost ($/kg)
	MWCNT	Carbon Black	Polymer	Bismalei-mide	Furfuryl-amine	
CNT(8)-CB(0)	8.0	0.0	0.7	1	1	11
CNT(6)_CB(2)	6.0	0.2	0.7	1	1	9
CNT(4)_CB(4)	4.0	4.0	0.7	1	1	7
CNT(2)_CB(6)	2.0	0.6	0.7	1	1	5
CNT(0)_CB(8)	0.0	0.8	0.7	1	1	4

[11] https://www.sciencedirect.com/science/article/pii/S0032386122008527#sec2

Cost of PEO - 5$
Cost of PDMS - 2$
Cost of PEO-PDMS can be approximated as - 3.5$
Changed of cost of composite - 0.7 × 3.5 = 2.45$
Increase in cost of composite if actual prices are taken = 2.45$ - 0.7$ = 1.75$

Let's assume that adhesive thickness is 1% of vessel radius and is 10 cm long. This gives us a polymer volume of 0.156 cc. The density of the composite is around 1 gm/cc. Which gives us a total weight of 0.156 gm. So the average cost of the polymer component would be around 1 Rs.

2. Adhesive

The calculations were done from this paper[12]. The cost prices of the raw material are given below:

Compound	Cost
Alginate	Rs 490/kg
PBS	Rs 6600/L
Dopamine Hydrochloride	Rs 1000/kg
NaOH	Rs 639/kg
NaIO4	Rs 4100/kg

Now, we do calculations to find the amount of each required. (The detailed calculations are not shown here to avoid confusion).

[12] https://www.pnas.org/doi/10.1073/pnas.1217972110#supplementary-materials

Material	Amount	Cost
Alginine	0.034 x g	Rs 0.0166 x
PBS	1.3 x mL	Rs 8.58 x
Dopamine Hydrochlroride	0.016 x g	Rs 0.016 x
NaOH	48.4 x µg	Negligible
NaIO4	0.0025 x g	Rs 0.01 x

Total: 8.6x Rs

Now to find x, let's assume that adhesive thickness is 1% of vessel radius and is 10 cm long. Then from calculations in the previous part, we get x = 0.12. Thus, we finally get the adhesive layer cost to be approximately Rs 1.

3. Biomolecule

For preparation of the last layer of our biosensor, a 2 µL of 100 U/mL[13] 3-HBDH enzyme solution is casted onto the polymer/SPCEs surface and is allowed to dry at 4°C. The solution prepared was from **'3-hydroxybutyrate dehydrogenase (3-HBDH) from *Pseudomonas lemoignei* lyophilized powder, ≥200 units/mg protein'** [14].

Units of enzyme required = 2 µL × 100 U/mL = $2 \times 10^{-6} \times 10^{5}$ U = 0.2 Units

Price of the very same powder (product code: H9408)[15] = ₹ 37,962.00 per 25 units
∴ Cost for 0.2 Units = ₹ (37,962 × 0.2)/25 = ₹ 303.69

[13,14] https://doi.org/10.3390/bios7040050
[15] https://www.sigmaaldrich.com/IN/en/product/sigma/h9408

CONCLUSION

A novel design of a biosensor was proposed that involved an adhesive layer glued to a blood vessel on top of which was a transducing polymer that would receive signals intercepted by a receptor biomolecule.

Prostate cancer was selected due to the potential for better screening and diagnosis methods, as well as the ability to predict individuals at higher risk. Its significant impact and the necessity for real-time monitoring also contributed to this choice.

After considering all the criteria needed for polymer for the biosensor, the PEO-PDMS copolymer was finalized. The polar side bonds with the adhesive side and the non-polar side bonds with the biomolecule.

3D-hydroxybutyrate was chosen as the biomolecule. 3D-hydroxybutyrate, found in higher levels in prostate cancer, is detected by an enzyme called 3-hydroxybutyrate dehydrogenase. This could help track and manage the disease.

The adhesive gel we chose was Adhesive hydrogel which is 10-100 μm thick gel film synthesized based on alginate, a polysaccharide and catechol.

The PSA test typically costs about 1000 Rs per test. For someone at a high risk, this test is recommended monthly for 10 years, resulting in a total cost of 120,000 Rs.

In comparison, the biosensor we're suggesting will cost approximately 300 Rs for a one-time installation. Consequently, in the long run, the biosensor will be **400 times more cost-effective** than the PSA test.