Queensland University of Technology
Brisbane Australia

PARTICIPANT INFORMATION FOR QUT RESEARCH PROJECT
– INTRODUCTION –

Cancer and Heart Disease Detection is A Spitting Distance Away

QUT Ethics Approval Number 1400000617

RESEARCH TEAM

Principal Researcher:	Associate Professor Chamindie Punyadeera	Queensland University of Technology (QUT)
Associate Researchers:	Associate Professor Karam Kostner	Mater Hospital
	Associate Professor John Atherton	Royal Brisbane Women's Hospital (RBWH)
	Associate Professor Chris Perry	Princess Alexandra Hospital (PAH)
	Professor Ian Frazer	University of Queensland (UQ/TRI)
	Professor Henry Krum	Monash University (MU)

DESCRIPTION

Many cancer causing agents (carcinogens) including nicotine, alcohol and human papilloma virus (HPV) enter the body through the mouth and throat. These carcinogens are implicated in many Head and Neck Cancers (HNC). Currently there are no reliable tests to detect these cancers at an early stage except for actual biopsy of the lesion. Cardiovascular diseases (CVD) including heart, stroke and blood vessel diseases affect a significant portion of the population. Recent studies have shown that early treatment with statin in CVD participants with hyperlipidemia, (low LDLs, high HDLs, low triglycerides and elevated blood C-Reactive Protein levels) significantly reduces cardiovascular risk, even in people without a history of CVD. However, nearly half of all first CVD events occur in people whose low-density lipoprotein (LDL) cholesterol levels are below current thresholds for lipid-lowering therapy. Therefore, further studies to refine our ability to identify people who are at risk of CVD are highly warranted.

Human saliva is a good diagnostic fluid as it mirrors biomolecules that are found in blood and about 20% of the molecules in blood are also present in saliva. This is further supported by our preliminary studies that showed the expression of various biomolecules associated with HNC and CVD in blood and saliva. The aim of the study is to develop a non-invasive diagnostic test using saliva to detect HNC and CVD at an early stage. Early detection and therefore, early intervention will lead to significantly reduced morbidity and mortality associated with these diseases. In the future, salivary markers can offer an attractive option for easy diagnosis rather than a blood test or surgical biopsy. Spitting into a cup or chewing onto a diagnostic test strip could one day be an alternative to having a finger prick or blood drawn at the doctor's office for the diagnosis of HNC and CVD.

You are invited to participate in this project as healthy controls to help define the limits of "normal". Our investigators will learn about the disease-specific biomolecules by comparing the participant group to the healthy control group.

PARTICIPATION

Participation will involve completing a questionnaire that will take approximately 5-10 minutes of your time. Questions will include personal details and health status. After finishing the questionnaire, you will be asked to give some saliva, urine and blood. Saliva and urine sampling will be carried out by trained professional staff/student from QUT or UQ. Blood will be collected by a trained nurse/phlebotomist.

Your participation in this project is entirely voluntary. If you agree to participate you do not have to complete any question(s) you are uncomfortable in answering. Your decision to participate or not will in no way impact upon your current or future relationship with QUT, Mater Hospital, RBWH, PAH, UQ or MU. If you do agree to participate you can withdraw from the project at any time without comment or penalty. Any identifiable information already obtained from you will be destroyed.

EXPECTED BENEFITS

It is expected that this project will not directly benefit you. However, it will lead to insights into the feasibility of using saliva as a non-invasive diagnostic fluid for heart diseases and cancer.

RISKS

The only foreseen risk associated with the study is discomfort during saliva, urine and/or blood collection. There will be no serious complication relating to saliva and urine collection. It is very rare for a blood collection to result in serious complications; however, there is a very small possibility of complications arising. These include infection, excessive bleeding, bruising, hematoma, fainting and dizziness. The risk is highly unlikely because trained professionals are collecting the samples in a safe and regulated environment. You will also need to fill out a questionnaire to provide information about the quality of the sample and provide information important for analysis of the study. There is only the inconvenience of filling out a short questionnaire.

CRITERIA FOR THE SELECTION OF HEALTHY PARTICIPANTS

There are certain criteria for you to become eligible to take part as a participant in this study.

1. You should refrain from consuming food or drink (except water) at least 2 hours prior to each test session. This includes also refraining from chewing gum.
2. To take part in this study you should have a good general state of health and should be aged between 18 and 100 years.
3. No fever or signs and symptoms suggesting active infection/illness on the day of saliva donation.
4. No mouth ulcers, inflammation of the gums, halitosis, any mouth infection, dry mouth or sensitive teeth on the day of intended donation of saliva.
5. No history of Hepatitis A or B.
6. No ear-nose-throat complaints.
7. Not undergoing dental treatment
8. Not wearing dentures.
9. No history of diabetes or hypoglycaemia.
10. No history of allergies including food allergies.
11. Not pregnant, planning to become pregnant in the near future or breast-feeding.
12. Not on medication such as lipid-lowering drugs, hormonal-replacement therapy, and evidence of hepatic dysfunction or supplements other than contraceptives.
13. A recent history of alcohol or drug abuse or other medical condition.
14. No prior history of any cancer. Participants with family history of cancer however, can be included.
15. No previous irradiation to head and neck region.
16. Available for one follow-up if and when needed.

Inclusion criteria for smoker controls (including ex-smokers) include the above mentioned inclusion criteria as well as the following:

17. Start and end date of cigarette/tobacco smoke
18. The number of cigarette smoked a day
19. Alcohol consumption i.e the number of beers/glasses of wines a week
20. Family history of cancer/CVD

Participation in other studies:
Participants of the present study are allowed to take part in other trials, provided the aforementioned exclusion criteria are adhered to.

STUDY SCHEDULE

Each session is expected to last for about 10 minutes, and it is anticipated that you participate in up to 6 sessions with a maximum of 2 sessions per single day. If you decide that you do not wish to come for scheduled visits please inform the study coordinator at least a day in advance if possible.

Before each saliva sample is collected you will be asked to refrain from food and beverages consumption for at least 2-hours with the exception of drinking pure water.

Visits are planned as follows:

1. Visit 1 (up to 10 mins):
   - Check your eligibility
   - Explain study details and activities involved
   - Provide participant information and consent pack
2. Visit 2 (10 mins):
   - Sign informed consent
   - Participant training
   - Saliva sample collection
   - Check adverse events
3. Subsequent visits (10 mins):
   - Saliva sample collection
   - Check adverse events

PROCEDURES

During saliva donation sessions you will be asked to donate saliva into collection tubes provided by us. At the time of saliva collection, a blood sample will also be taken. Human saliva will be collected using the standard (A) "Drool" method, (B) Versi.Sal(R) Absorbent pads, (C) Super.Sal(R) Absorbent pads, (D) DNA.Sal(R) cell scrapers, (E) Mechanically stimulated saliva, (F) Acid stimulated saliva and (G) Swish and gargle with saline and/or sucrose mouthwash. Urine will be collected into Urine cups.

For participants with salivary gland deficiencies, a stimulation regime will be deployed, i.e. (E) Mechanical stimulation and/or (F) Acid stimulation.

(A) The Drool Method:

1. You will be asked to refrain from eating and drinking for 1-hour prior to the collection of saliva, with the exemption of drinking plain water to ensure they are fully hydrated.
2. 5 minutes prior to collection, the mouth should be rinsed with water. During saliva collection/expectoration, you will sit comfortably in an upright position with head slightly tilted forward so that saliva pools to the front of the mouth. Collection will be done under supervision/assistance of the trained staff.
3. Pool saliva (head tilted slightly down) in the mouth for about 2-5 minutes, and expectorate into a "Specimen" collection cup (to get at least 1-5 ml of saliva).
4. Collected human saliva will be labelled and immediately frozen on dry ice.

(B) The Versi.Sal (TM) method:

1. You will be asked to 'pool' saliva in the mouth, under the tongue to facilitate faster collection times.
2. The absorbent pad material end of the Versi.SAL collection device will be placed UNDER the tongue

a. Do NOT chew or suck the collection pad
b. Continue to collect oral fluids (Approximately 1-2 minutes) until the sample volume indicator changes, ie when the blue coloured line disappears.

3. Versi.Sal will then be collect by the research staff for further processing.

(C) The Super.Sal (TM) method:

1. You will be asked to 'pool' saliva in the mouth, under the tongue to facilitate faster collection times.
3. The absorbent pad material end of the Super.SAL collection device will be placed along the SIDE of the tongue

a. Do NOT chew or suck the collection pad
b. Continue to collect oral fluids (Approximately 1-2 minutes) until the sample volume indicator changes, ie when the blue coloured line disappears.

3. Super.Sal will then be collect by the research staff for further processing.

(D) The DNA.Sal (TM) method:

1. You will be provided with a DNA.Sal collection device.
2. The DNA.SAL device will be placed in mouth with the collection teeth perpendicular to the inside of the cheeks towards the lower end of the inside of the cheeks where the cheeks meet the gum line
3. A finger should be placed on the OUTSIDE of the cheeks to act as resistance while collecting the sample. The collection teeth of the device should be raked along the inside of the cheeks until the collection area is sufficiently loaded with saliva and cellular material.
4. Recommended raking time is 30 seconds to one minute and hand the DNA.SAL back to the research staff.

(E) Mechanically stimulated saliva:

1. If possible, you will be asked to refrain from eating and drinking for 1-2 hours prior to the collection of saliva, with the exemption of plain drinking water to ensure they are fully hydrated.
2. 5 minutes prior to collection, the mouth should be rinsed with water. During saliva collection/expectoration, you will sit comfortably in an upright position with head slightly tilted forward so that saliva pools to the front of the mouth. Collection will be done under supervision/assistance of a trained experimenter.
3. Pool saliva (head tilted slightly down) in the mouth for 1-minute, and expectorate into plastic container for disposal.
4. Chew flavourless chewing gum or sterilised paraffin film for 30 seconds with head tilted slightly down; allow saliva to pool to front of mouth. Expectorate into plastic container for disposal (while keeping the chewing gum in mouth).
5. Chew flavourless chewing gum or paraffin for another 30 seconds with head tilted slightly down, allow saliva to pool to front of mouth. Expectorate into pre-labelled sterile collection plastic container every 30 seconds for total time up to 5 minutes to get at least 2 ml of saliva.

(F) Acid stimulated saliva:

1. If possible, you will be asked to refrain from eating and drinking for 1-2hours prior to the collection of saliva, with the exemption of plain drinking water to ensure they are fully hydrated.
2. 5 minutes prior to collection, the mouth should be rinsed with water. During saliva collection/expectoration, you will sit comfortably in an upright position with head slightly tilted forward so that saliva pools to the front of the mouth. Collection will be done under supervision/assistance of the trained experimenter.
3. Pool saliva (head tilted slightly down) in the mouth for 1-minute, and expectorate into plastic container for disposal.
4. Use lemon lollies to stimulate saliva production if you are having difficulties to produce saliva in an unstimulated manner.
5. Pool saliva in front of mouth and gently expectorate every 30 seconds into labelled designated for disposal.
6. Pool saliva in front of mouth and gently expectorate every 30 seconds into tubes for collection until 2 ml of saliva is collected.

(G) Swish and gargle with saline and/or sucrose mouthwash:

1. You will be asked to refrain from eating and drinking for 1-hour prior to the collection of saliva, with the exemption of drinking plain water to ensure they are fully hydrated.
2. Oral rinse samples will be collected by using a 30 second alternating oral swish and gargle of 10 ml of 0.9% saline solution. You will be asked to alternate between swish and gargle every 5 seconds and expectorate the sample into a sterile collection tube.

All saliva samples will be transported on dry ice and stored at QUT according to local storage procedures and protocols.

PRIVACY AND CONFIDENTIALITY

Saliva, blood and urine samples will be stored for no longer than 15 years for further characterisations. We may use the biological samples for future research work. Tissue and/or blood and/or saliva collected for this study may be used to establish of a cell line which will remain the property of the research team. I understand there will be no financial benefit to me if a cell line is created using my tissue. No cell lines or any other kinds of replication of the whole biological samples will be created.

All comments and responses will be treated confidentially unless required by law. Any data collected as part of this project, including salivary flow rate and potential biomarkers status in the biological samples, will be stored securely as per QUT's Management of research data policy.

The project is funded by Queensland Government, QUT, Garnett-Passe and Rodney Williams Foundation and they will not have access to the data obtained during the project.

CONSENT TO PARTICIPATE

The return of the completed questionnaire and consent form is accepted as an indication of your consent to participate in this project.

QUESTIONS / FURTHER INFORMATION ABOUT THE PROJECT

If you have any questions or require further information please contact the researcher below.

Associate Professor Chamindie Punyadeera chamindie.punyadeera@qut.edu.au

CONCERNS / COMPLAINTS REGARDING THE CONDUCT OF THE PROJECT

QUT is committed to research integrity and the ethical conduct of research projects. However, if you do have any concerns or complaints about the ethical conduct of the project you may contact the QUT Research Ethics Unit on 07 3138 5123 or email ethicscontact@qut.edu.au. The QUT Research Ethics Unit is not connected with the research project and can facilitate a resolution to your concern in an impartial manner.

Thank you for helping with this research project. Please keep this sheet for your information.

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PARTICIPANT INFORMATION FOR QUT RESEARCH PROJECT
– QUESTIONNAIRE FOR HEALTHY PARTICIPANTS –

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Name of participant: .....

Date of birth: ..... **Gender:** ☐Male ☐Female

Address: .....

.....

Please tick

Height (cm):	☐<149	☐150-159	☐160-169	☐170-179	☐180-189	☐>190	☐unknown
Weight (kg):	☐<50	☐60-69	☐70-79	☐80-89	☐90-99	☐>100	☐unknown

Q1.	Are you between the age of 18 and 100?	☐YES	☐NO
Q2.	If you are currently working, what is your occupation?	.....	
Q3.	Are you now or have you ever been a smoker?	☐YES	☐NO
Q4.	If you answered "YES" to Q3, do you still smoke cigarettes/nicotine/tobacco?	☐YES	☐NO
Q5.	If you answered "YES" to Q3, how many cigarettes per day do you or did you smoke?	.....	
Q6.	If you answered "YES" to Q3, at what age did you start smoking?	.....	
Q7.	If you answered "NO" to Q4, at what age did you quit smoking?	.....	
Q8.	Do you drink alcohol?	☐YES	☐NO
Q9.	If you answered "YES" to Q8, how much and how often do you drink in a week?	.....	
Q10.	Are you suffering from any of the following:		
	Cold sores, mouth ulcers, or oral infection	☐YES	☐NO
	Periodontal disease or gingivitis	☐YES	☐NO
	Autoimmune	☐YES	☐NO
	Any infectious diseases	☐YES	☐NO
	Musculoskeletal problems	☐YES	☐NO
	Recent operation or trauma	☐YES	☐NO
	Allergies (including food allergies)	☐YES	☐NO
	Diabetes	☐YES	☐NO
	Hypoglycaemia	☐YES	☐NO
	Ear-Nose-Throat complaints	☐YES	☐NO
Q11.	Do you have family history of cancer or cardiovascular diseases (CVDs)?	☐YES	☐NO
Q12.	If you answered "**YES**" to **Q11**, please provide details of relative/s and type of cancer/CVD?	.....	
Q13.	Are you currently on any medication/treatment?	☐YES	☐NO
Q14.	If you answered "**YES**" to **Q13**, please document the type of treatment	.....	
Q15.	If female, are you pregnant (to the best of your knowledge)?	☐YES	☐NO
Q16.	Are you presently suffering from, or during the past 7 days have you suffered from, a cold or flu?	☐YES	☐NO
Q17.	Are you undergoing dental treatment?	☐YES	☐NO
Q18.	Do you wear dentures?	☐YES	☐NO

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CONSENT FORM FOR QUT RESEARCH PROJECT

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RESEARCH TEAM CONTACTS

Associate Professor Chamindie Punyadeera chamindie.punyadeera@qut.edu.au

STATEMENT OF CONSENT

By signing below, you are indicating that you:

- Have read and understood the information document regarding this project.
- Have had any questions answered to your satisfaction.
- Understand that if you have any additional questions you can contact the research team.
- Understand that you are free to withdraw at any time, without comment or penalty.
- Understand that you can contact the Research Ethics Unit on 07 3138 5123 or email ethicscontact@qut.edu.au if you have concerns about the ethical conduct of the project.
- **Agree not to restrict the use of data generated using my samples.**
- **Agree to the use of my tissue and/or blood and/or saliva for future research, and the establishment of a cell line which will remain the property of the research team. I understand there will be no financial benefit to me if a cell line is created using my tissue.**
- Agree to participate in the project.

Name _____

Signature _____

Date _____

Please return these sheets to the investigator.