ORIGINAL ARTICLE

Novel Transfer of Very-Low Viscosity Ultraviolet Light Curable Cyan Methacrylate on Saline Immersed In-Vitro Sheep Heart Model and Paintbrush Technique

Nueva transferencia de cianometacrilato de muy baja viscosidad curado por luz ultravioleta en un modelo in vitro de corazón de oveja inmerso en solución salina y la técnica de pincelado

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ABSTRACT

Background: Cyan methacrylate is a compound with remarkable adhesion properties used in different specific areas, including medicine, where it can be applied in some entities and is also subject of research in the field of cardiology
Objective: The aim of this study was to develop a novel technique for the transfer of very-low viscosity cyan methacrylate on the inner and outer surfaces of the heart.
Methods: Very-low viscosity ultraviolet curable cyan methacrylate was mixed with commercially available dye to demonstrate its ability of transfer on the inner and outer surfaces of an in-vitro heart model. Cyan methacrylate (0.5 ml) was mixed with 0.2 ml of ink, and the material was injected on the surface of the heart in dry air, allowing its fixation for 2-3 seconds. Subsequently, the whole preparation was immersed in saline, and was vigorously shaken to remove the unbound compound. A similar experiment was performed without cyan methacrylate, and with high viscosity cyan methacrylate (3000 cps). A significant amount of the compound was visually found to attach to the surfaces of the heart compared with ink alone. Then, after soaking the tissue in saline solution, effective transfer of the compound was assessed on the inner and the outer surfaces of the heart.
Results: Among various techniques, the paintbrush technique was the most effective one for the targeted transfer of the compound. With this technique, it was easier to transfer the compound on the epicardial and endocardial surfaces. Also, other specific areas, such as the left atrial appendage, the left ventricular inner surfaces at the origin of the papillary muscle and the left ventricular apex were successfully investigated. The ultraviolet light source was a pen-shaped device based on a light emission diode. Some compound precipitation was observed in some areas following ultraviolet treatment. The experiment was repeated with three different samples to determine the results.
Conclusions: Very-low viscosity ultraviolet curable cyan methacrylate transfer is potentially useful to study the inner and outer surfaces of the heart. This technique could be useful for growth factor transfer.

Key words: Cyan methacrylate - Low viscosity bio-adhesive - transfer technique - cardiac surfacesors or protein molecules on the surface of the heart.

RESUMEN

Introducción: El cianometacrilato es un compuesto con propiedades excepcionales de adhesión que se utiliza en diversos campos específicos incluida el área médica, donde encuentra aplicación en algunas entidades y asimismo es objeto de investigación en el terreno de la cardiología.
Objetivo: Desarrollar una nueva técnica para la transferencia de cianometacrilato de muy baja densidad sobre las superficies internas y externas del corazón.
Material y métodos: Se combinó el cianometacrilato curado por luz ultravioleta de muy baja densidad con colorante comercial para demostrar su capacidad de transferencia sobre las superficies externas e internas de un modelo de corazón in vitro. Se mezclaron 0,5 ml de cianometacrilato con 0,2 ml de tinta, y el material se inyectó sobre la superficie del corazón en aire seco, permitiendo su fijación durante 2-3 segundos. A continuación, toda la preparación se sumergió en solución salina y se agitó vigorosamente para eliminar el compuesto no ligado. Se realizó un experimento similar sin cianometacrilato y con cianometacrilato de alta viscosidad (3.000 cps). Se comprobó visualmente después del lavado que una cantidad significativa del compuesto se liga a las superficies del corazón en comparación con la tinta sola. Luego se investigó la transferencia efectiva del compuesto a las superficies internas y externas del corazón después de haber sumergido el tejido en solución salina.
Resultados: Entre diversas técnicas, la de pincelado fue la más efectiva para la transferencia orientada del compuesto. Mediante esta técnica fue muy fácil transferir el compuesto sobre las superficies endocárdica y epicárdica. También otras áreas específicas, como la orejuela auricular izquierda, las superficies internas del ventrículo izquierdo en el origen del músculo papilar y el ápex ventricular izquierdo, fueron investigadas exitosamente. La fuente de luz ultravioleta fue un dispositivo en forma de lapicera basado en un diodo emisor de luz. Luego del tratamiento con luz ultravioleta se observó cierta precipitación del compuesto en algunas zonas. El experimento se repitió con tres muestras para determinar el resultado.
Conclusiones: La transferencia de cianometacrilato de muy baja viscosidad curado por luz ultravioleta es potencialmente útil para el estudio de las superficies internas y externas del corazón. Esta técnica podría servir para la transferencia de factor de crecimiento.

Palabras clave: Cianometacrilato - Bioadhesivo de baja viscosidad - Transferencia técnica - Moléculas de proteína sobre la superficie del corazón

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Abbreviations

CMA Cyan methacrylate	**VEGF** Vascular endothelial growth factor

INTRODUCTION

Cyan methacrylate (CMA) is used as a compound with exceptional adhesion properties in various specific fields. In the medical setting, CMA is used in gastroenterology to induce thrombosis in esophageal varices. (1) It is also used for closure of anal (2), pancreatic (3) and esophageal-pancreatic fistulas. (4) Embolization, leading to septic and embolic complications has been previously reported. (5, 6) However, these complications are only seen in a minimal number of cases. The viscosity of CMA varies and hence its clinical application. It rapidly oxidizes in contact with saline. Moreover, CMA has many non-medical uses. Upon contact with blood, it is rapidly oxidized, activating fibrin and the coagulation cascade. Myocardial distribution of drugs can be achieved by its local application on the epicardium, which is dependent on capillary vessels. (7) Hence, the application of CMA needs to be cautious to achieve maximum benefits with minimal side effects. It has been used to close pericardial effusion during percutaneous aortic valve implantation. (8) However, there are many scientific challenges in the therapy of certain complex lesions, as percutaneous mitral regurgitation therapy, left atrial appendage closure and complex myocardial tissue engineering. In this study, CMA Cyberbond U301 and U303 were investigated for their potential transfer on myocardial surfaces. The purpose of this study was to evaluate the transfer of CMA on the surfaces of the heart, with the primary aim of developing a novel method of percutaneous transfer of very-low viscosity CMA and its potential conjugates in the future. The outcome would support the future design of hydrogels with similar viscosity.

METHODS

This study was focused on CMA transfer on the heart surfaces. Low viscosity (40±10 cps and setting time <3s, specific gravity 1.1g/cc) injectable CMA was obtained from Cyberbond TM (U 301, Figure 1). Cyan methacrylate (0.5 ml) was mixed with 0.2 ml of commercially available writing ink in a plastic syringe, and transferred by needle injection in the air on the surfaces of the heart. The transfer was performed by a spraying method. The resultant compound (CMA + ink) was allowed to set on the surfaces of the targeted regions of the heart for about 3 seconds, and was immediately vigorously washed in saline. The amount of compound attached to the surface was quantified visually. As control, the amount of ink alone attached on to the surface was simultaneously analyzed in a similar experiment.

After that, the experiment was also performed in saline with the paintbrush technique. The paintbrush bristles were very thin cattle hairs. The bristles were soaked in the compound (Cyberbond U301), and the simple painting stroke and contact method was followed, each single stroke having a contact time of about 3 seconds. Then, another cardiac target area was chosen, and the technique was applied again. Some of the target areas were the endocardial and epicardial surfaces of the ventricles and atria, and the endocardial surfaces and the left atrial appendage.

Fig. 1. A. Ultraviolet light source with a 1.5 cm diameter pen-shaped device (9 LED UV Mode blacklight flashlight). **B.** Cyan methacrylate tube.

The experiment was repeated with other techniques like application with a cotton bud soaked in CMA, through spraying in the proximity of the tissues with a thin needle, and also by bolus syringe spraying without needle through direct contact with cardiac tissues. The compound was then applied on the external surfaces of the heart. This experiment was essayed on three different sheep heart models, and the results were analyzed.

After that, the experiment was similarly repeated with CMA (Cyberbond U303), with higher viscosity of about 3000±1000 cps (specific gravity 1.1g/cc and setting time <4s). The Ultraviolet light source used in the study was a 1cm diameter pen-shaped 9 LED light-based UV device in the range of 385-400 nm.

RESULTS

Among the various techniques with different sheep cardiac samples, the paintbrush technique showed the maximum amount of CMA transfer on the cardiac surfaces. Other techniques like CMA transfer using a cotton bud, spraying with thin needle and syringe on nearby tissues and direct spraying on the surfaces through syringe tips showed only a negligible amount of transfer by visual observation, i.e., less than 20% compared with the paintbrush technique.

The amount of CMA transfer in the air was significant, and it was well visualized when the test was per-

formed in the air (Figure 2). Figure 3 demonstrates the application of the compound on the right (Image A) and left (Images A and B) ventricular surfaces of the heart after saline immersion of the sheep heart preparation. The successful transfer of the compound was seen in all the sheep heart models. Figure 4 shows control with ink alone (Image A) in the air and ink alone in the saline-immersed model (Image B). The control images compared with the images in the air and the saline-immersed sample showed very negligible colors attached on to the ventricular surfaces.

Saline immersion reduced the amount of compound attached to the surface of the heart. Compared with air transfer, upon saline immersion, the quantum of transfer was less. This is due to the dissolution of the compound and to the liquid interface during transfer. In the air, the compound fixes easily in a few seconds. Though the amount of transfer was relatively less with saline interfacing, the smell of CMA was predominant on the surfaces. A similar study was performed with the higher viscosity (3000 cps) Cyberbond U303 (Figure 5, images B and C).

Using the more viscous CMA U303 the amount of transfer was minimal. By direct visual assessment, this was approximately 20% of the very low viscosity U301 compound. This is predominantly due to the greater CMA U303 viscosity, hampering its withdrawal with a needle. By the smelling method, a very significant amount of transfer was observed with CMA U301, with lower viscosity than the U303 compound. When the UV treatment was applied for a few seconds after transfer on the endocardial and epicardial surfaces, micro precipitates formed on the surfaces. This is due to crosslinkage of polymers induced by UV exposure, which was obtained by immersing the UV device in saline and activating it for few seconds.

DISCUSSION

Amount of transfer in air and saline

This in-vitro study demonstrates the transfer of CMA on the heart surfaces. The initial results based on a limited number of experiments show that the transfer was very effective in the air and relatively less in the saline-immersed sample. This is due to direct interference with the saline interface when CMA makes contact with tissues. However, though the quantum of transfer was less, it was reasonably visualized on the surfaces. In-vivo, the endothelial surfaces have natural adhesion molecules and receptors, as E-selectin receptors that can absorb the compound better than in-vitro experiments. There are other adhesion molecules like ICAM-1, (9, 10) and Beta-2 integrin. (11). Thus, better results could be expected in-vivo.

Direct/thin needle injection sprays, cotton bud and paintbrush techniques

Low viscosity CMA has the advantage of being used with needle. However, needle injections through sharp needles have the risk of cardiac perforation. Spraying through direct syringe manipulation, which is a form of spraying, is not easy to perform inside the cardiac chambers. Spraying depends on the distance

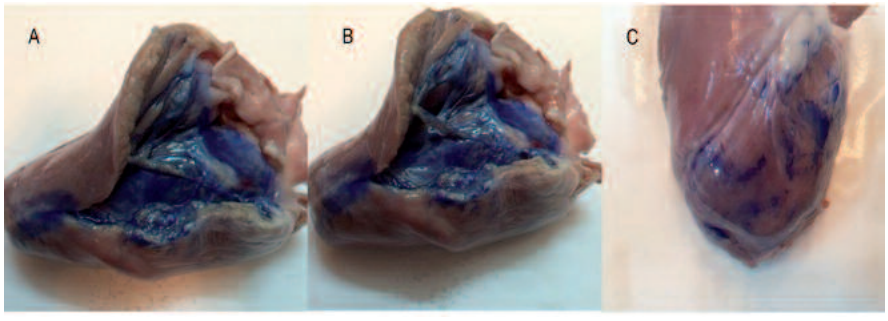

Fig. 2. Transfer of cyan methacrylate on the inner surface of the heart in air (Images **A** and **B**). Image **C** shows transfer of cyan methacrylate on the outer surface of the heart.

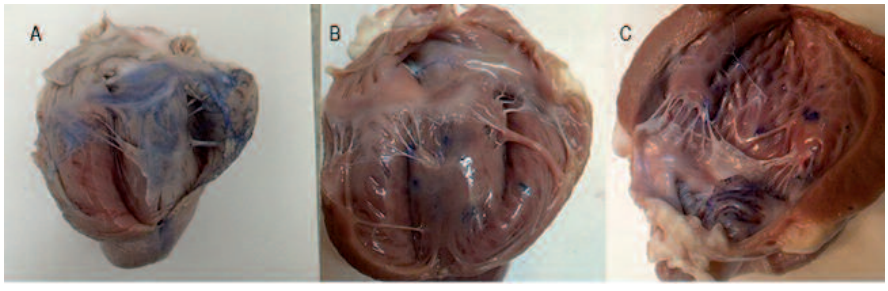

Fig. 3. Transfer of cyan methacrylate on the inner surface of the heart in the saline immersed sheep heart model. Image **A** shows the inner surface of the right heart. Images **B** and **C** show transfer of cyan methacrylate on the left ventricular surface of the sheep heart model.

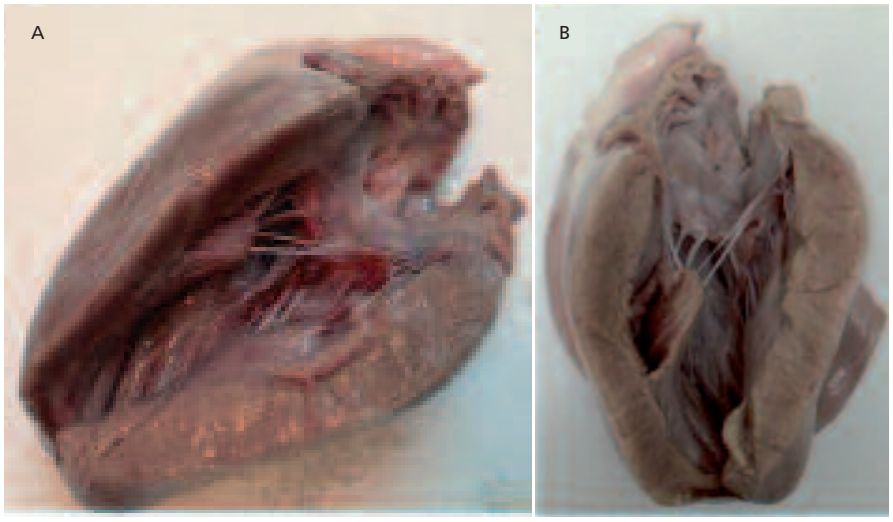

Fig. 4. Control images. Image A shows the result after transfer in air of ink alone and washed in saline. Image B shows transfer of ink alone performed in the saline immersed model.

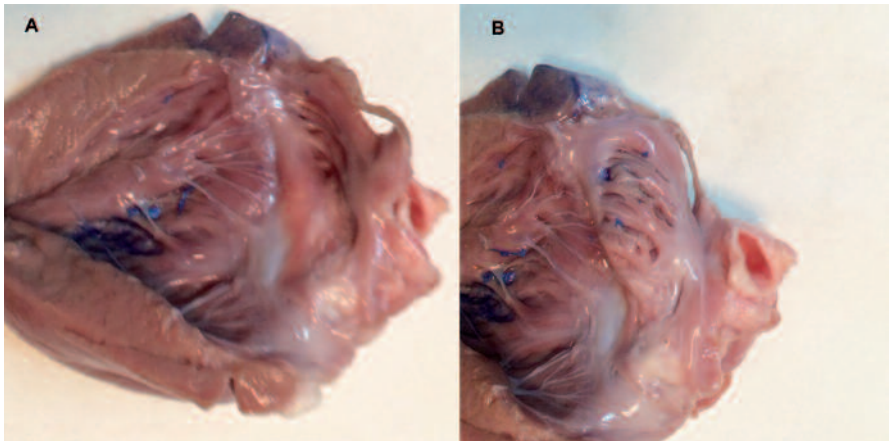

Fig. 5. Images showing transfer of the more viscous cyberbond U303 on the inner surfaces of the heart.

between the device and the tissues, the root mean square deviation from the center (δ) to the sprayer, the spray velocity and the angular coordinate (cosine θ) between the sprayer and the target surface. (12) Painting involves a single coordinate, the brush angle and the contact; whereas spraying involves multiple coordinates (δx, δy, δz), which are largely affected by the interface distance and molecular movements. Hence, paintbrush could be the technique in which cardiac perforation would be negligible, and also the easiest to work on selected targets. In addition, it was seen that this compound does not clog the brush bristles even if it was applied on the bristles for many seconds. As the viscosity of the compound is very low, its use is expected to cause embolism (Poiseuille's equation). Theoretically, however, as the dilution is high, the embolism due to the diluted material would be very insignificant. The ultraviolet source is a pen-shaped device (see Figure 1), which is based on a light emission source. The device was immersed in saline and exposed to the transferred sample for a few seconds, forming micro precipitates on the surface of the transferred sample. These micro precipitates could result in embolism. Hence, ultraviolet light treatment on the endothelial surface may not be a good method. However, on the external surfaces of the heart, this could help to form tissue or vascular proliferation based on the conjugate attached to CMA. Since CMA is used for various biological procedures it is biocompatible, and also hemocompatible. (13) These observations are preliminary, and real-time in-vivo experiments need to be performed to demonstrate the capabilities of the transfer method as well as the potential benefits of the compound in tissue culture and in-vivo experiments.

Percutaneous method

The experiments were performed basically focussed on future percutaneous modeling. Compounds like vascular endothelial growth factor and fibroblast

growth factors can be effectively conjugated with CMA with or without streptavidin and functional groups. Also, pluronic conjugation could be very interesting to study in the future. Cyan methacrylate could be washed with acetone if it needs to be washed out or eliminated from tissues.

Adhesion kinetics, brush mechanics and paint transfer

Low viscosity adhesives have free motion of the polymer particles and more Van der Waals forces. When the viscosity is high, the molecular motion is lower and thereby less Van der Waals force is exerted. The London dispersion forces also tend to be greater, as they are inversely proportional to the molecular contact distances. (14) The transfer of compound is also dependent on the angle of contact, the kinetic energy during transfer and the presence of non-cross chained and short-chained polymers. (14, 15) All these parameters are higher with the paintbrush technique as this has variable angles of contact and higher kinetic energy during transfer compared with other techniques. Low viscosity adhesives tend to have smaller and reduced cross-chained molecules. These are the possible explanations for the observations in the study.

Polymer viscosity and implications

Cyan methacrylate adhesion on the substrate and conjugate release depend on viscosity. This is explained by the Hagen-Poiseuille equation: when the viscosity increases the rate of release of drugs/conjugate (dM/dt) is slower. (16, 17) Hydrogels swell when exposed to water. This swelling results in increased viscosity and therefore the adhesion to the substrate would be lower. (16) Previoss studies with hydrogels have shown transfer of drugs or conjugates to tissues. Most of these studies have used higher viscosity (1000 to 3000 cps) hydrogels or the viscosity is not well defined in the studies. (18-24) Certain alginates are used as biological carriers; however, they also have higher viscosity. (23) Gecko, a bioadhesive, has greater viscosity. (25) Therefore, lower viscosity is very much preferable than higher viscosity in the setting of blood interfacing during transfer. Hence, in this study, very-low viscosity explains the difference in transfer of U301 (cyberlite) and U303 compounds.

Mathematical modeling of paintbrush

The frictional energy at brushing is given by the formula $E_{frict} = \mu \sum F \cdot (\kappa f \ \Delta x_{par} + (1-\kappa f) \cdot \Delta x_{perp} \cdot)$, where F is force, x is the dragging vector (parallel or perpendicular), and μ is the frictional coefficient. (26) The bend energy of the brush is bend energy $(\theta) = k\ (\theta - \rho)3$, where $\rho = \min(\theta', \alpha)$ and α is the plasticity value. (24) The user interface i.e. the saline/blood interface will modify the transfer of compounds on to the substrate. An ideal paintbrush has to be modeled based on the surface area, height and bend characteristics of the bristles for this specific purpose, so that it is suitable for a percutaneous approach in the future. (26, 27)

Hydrogels and Cyanmethacrylate

Hydrogels are commonly used as bioadhesives. Hydorgels can be suitably modified to achieve low viscosity. However, most current studies use hydrogels as carriers of biological compounds. (18-22) In most of the studies the route of injection is surgical, such as ligation of the left anterior descending coronary artery and intramyocardial injection. However, this study was performed with the intention of modeling a novel percutaneous technique in the future. Hence, very-low viscosity was studied. Direct intramyocardial injection is not easily feasible in the real life setting, as it requires a surgical pathway, and is, therefore, logistically difficult.

Cyanmethacrylates can be conjugated with VEGF or other growth factors with thiolene reaction or introduction of a –SH functional group, which is more stable and easier than –OH groups. (28, 29) Also, this would not change the viscosity as it would maintain the straight link polymer nature, not introducing cross-linkage of polymer chains.

Integrins

Conjugation with integrins during bioadhesion has shown better results in other studies. (30, 32) Cyanmethacrylate causes mild inflammation on the tissues, (33, 34) which would release P-selectin and E-selectin, resulting in better adhesion. Inflammation triggers inflammasome activity, and thereby inflammatory pathways and integrins are activated. (35) Hence, conjugation with RGD, or GFOGER would result in better adhesion of the compounds on tissue substrates. (36)

Theoretical internal endocardial to epicardial micro-bypass collaterals

Internal coronary bypass (epicardium-endocardium) collaterals could be created by painting the interventricular septum or right ventricular walls with conjugated VEGF. The capillary vascular perfusion of the septum is less than that of the ventricular walls. (7) Hence, this could be a potential target which needs angiogenesis. The left ventricle could be susceptible of embolisation. Therefore, the right ventricular approach could be safer, though this is currently speculative.

Other transfer compounds

At present, various other compounds are available, which could be used for further study. Certain biological hydrogels can be synthesized by modification of aldehyde-amines, and, thus the PEG-dextran tissue specific adhesion and viscosity could be modified. (37) These compounds are tissue compatible and have better biological properties than CMA. Gecko (38) is another biologically inspired tissue adhesive, which is

available for closure of atrial and ventricular septal defects, and has higher viscosity.

Overcoming air-tissue interface

This study has shown that transfer is better in air or dry surfaces than in saline. This can be mimicked in real time by creating an air-tissue interface using simultaneously carbon dioxide. This is because carbon dioxide is rapidly dissolved in plasma, and hence the chance of air embolism is very low. In the past, carbon dioxide was used for angioscopy though it was not very successful. (39)

Limitations

This study is an initial interesting observation of a novel technique. Hydrogels can be used for similar studies instead of CMA. These hydrogels with low viscosity are not commercially accessible at present. The concept, however, with CMA is interesting, as it is cost effective and it is available in needle-based injections. Cyan methacrylate low viscosity physical and setting properties allowed the compound transfer, complying with the paintbrush method. Further studies need to be performed with CMA, hydrogels, and Gecko adhesive to demonstrate the effects. Low viscosity CMA vaporizes, causing inhalatory tracheo-bronchitis to the research workers on inhalation. There could be concerns with CMA about the degradation products, such as cyanoacetones and formaldehyde, resulting in histotoxicity. (40) Hence, the study of a suitable hydrogel with similar mechanical properties would be better. This is an in vitro study, and additional studies need to be performed in animals with CMA and low viscosity hydrogel conjugates for further observation and validation.

CONCLUSION

Transfer of low viscosity, ultraviolet curable CMA on the inner and outer surfaces of the heart is potentially feasible and could be useful for the future transfer of growth factors or protein molecules on the heart surface.

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Conflicts of interest

None declared.
(See authors' conflicts of interest forms on the website/ Supplementary material)

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ICMJE Form for Disclosure of Potential Conflicts of Interest

Instructions

The purpose of this form is to provide readers of your manuscript with information about your other interests that could influence how they receive and understand your work. The form is designed to be completed electronically and stored electronically. It contains programming that allows appropriate data display. Each author should submit a separate form and is responsible for the accuracy and completeness of the submitted information. The form is in six parts.

1. Identifying information.

2. The work under consideration for publication.

This section asks for information about the work that you have submitted for publication. The time frame for this reporting is that of the work itself, from the initial conception and planning to the present. The requested information is about resources that you received, either directly or indirectly (via your institution), to enable you to complete the work. Checking "No" means that you did the work without receiving any financial support from any third party -- that is, the work was supported by funds from the same institution that pays your salary and that institution did not receive third-party funds with which to pay you. If you or your institution received funds from a third party to support the work, such as a government granting agency, charitable foundation or commercial sponsor, check "Yes".

3. Relevant financial activities outside the submitted work.

This section asks about your financial relationships with entities in the bio-medical arena that could be perceived to influence, or that give the appearance of potentially influencing, what you wrote in the submitted work. You should disclose interactions with ANY entity that could be considered broadly relevant to the work. For example, if your article is about testing an epidermal growth factor receptor (EGFR) antagonist in lung cancer, you should report all associations with entities pursuing diagnostic or therapeutic strategies in cancer in general, not just in the area of EGFR or lung cancer.

Report all sources of revenue paid (or promised to be paid) directly to you or your institution on your behalf over the 36 months prior to submission of the work. This should include all monies from sources with relevance to the submitted work, not just monies from the entity that sponsored the research. Please note that your interactions with the work's sponsor that are outside the submitted work should also be listed here. If there is any question, it is usually better to disclose a relationship than not to do so.

For grants you have received for work outside the submitted work, you should disclose support ONLY from entities that could be perceived to be affected financially by the published work, such as drug companies, or foundations supported by entities that could be perceived to have a financial stake in the outcome. Public funding sources, such as government agencies, charitable foundations or academic institutions, need not be disclosed. For example, if a government agency sponsored a study in which you have been involved and drugs were provided by a pharmaceutical company, you need only list the pharmaceutical company.

4. Intellectual Property.

This section asks about patents and copyrights, whether pending, issued, licensed and/or receiving royalties.

5. Relationships not covered above.

Use this section to report other relationships or activities that readers could perceive to have influenced, or that give the appearance of potentially influencing, what you wrote in the submitted work.

Definitions.

Entity: government agency, foundation, commercial sponsor, academic institution, etc.
Grant: A grant from an entity, generally [but not always] paid to your organization
Personal Fees: Monies paid to you for services rendered, generally honoraria, royalties, or fees for consulting , lectures, speakers bureaus, expert testimony, employment, or other affiliations
Non-Financial Support: Examples include drugs/equipment supplied by the entity, travel paid by the entity, writing assistance, administrative support, etc.
Other: Anything not covered under the previous three boxes
Pending: The patent has been filed but not issued
Issued: The patent has been issued by the agency
Licensed: The patent has been licensed to an entity, whether earning royalties or not
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Dr. Arokiaraj has nothing to disclose.

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