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Rigor and reproducibility in scientific research: Method section

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Outline

- The importance of Rigor and reproducibility in scientific research in Sciences
- Method Section: Proposal and Article
- Introduction to method section in a scientific article: Who, What, When, Where, How, and Why, Ethics and Statistical analysis
- Standards of reporting: ARRIVE Guidelines
- Examples
- Conclusion: Important recommendations

The Importance of Rigor and Reproducibility in Sciences

- For peer review: Manuscript acceptance
- Post peer review: Pubpeer
- Letter to Editor
- Retracted
- Scientific Integrity

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Anti-microbial/oxidative/inflammatory nanogels accelerate chroni… wound healing

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Biofilms

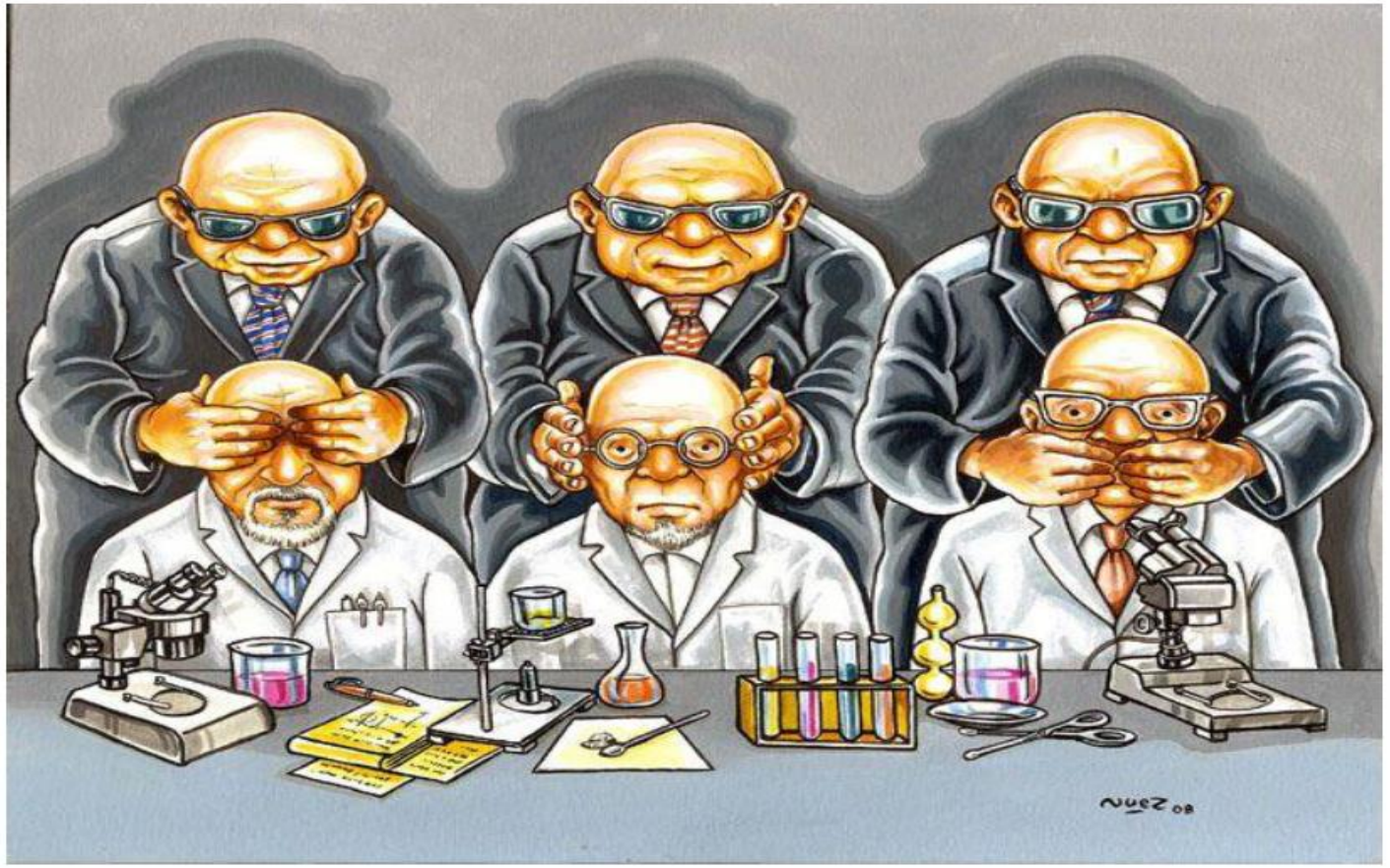
ABSTRACT

The most common cause of delayed h…g in chron… wounds is microbial pathogenesis, in which localized colonization can cause severe inflammation, …infection, and even sepsis in some cases. Towards this end, we have developed a m…tional nanogel possessing antimicrobial/oxidative/inflammatory characteristics for rapid wound healing. We in… …quercetin (Qu) to prepare carbonized nanogels (CNGs) through polymerization and mild ca…nization. …e Qu…CNGs with antioxidant activity were further used as templates to prepare multifunctional nano… co…ning copper sulfide (CuS) nanoclusters that possess superior catalytic and photoresponsive properties. Th… …imum inhibitory concentration of the nanogels (CuS/Qu–CNGs) towards tested bacteria …-folds low…han monomeric Qu or Qu–CNGs under NIR-II light irradiation. Furthermore, CuS/Qu–CN… demo…ated efficie… penetration into the extracellular biofilm matrix, resulting in eradication of methicillin…sistant *S… …occus …ureus* (MRSA) associated biofilm on diabetic mice wounds. The CuS/Qu–CNG… …ogels s…pressed …flammatory cytokines (IL-1β) in the infectious wound sites and regulated the expression o… …ammatory IL-10 and TGF-β1 during and after recovery from infection, respectively. Along with *in vivo* bacte…al effects, the CuS/Qu–CNGs promote angiogenesis, epithelialization, and collagen synthesis to… …celerate wound h…ng. Faster wound healing was attributed to the triple features (i) antioxidant Qu-CNGs to… …d the pathogen-ind…ed oxidative stress, (ii) enhanced bacterial contact due to polyphenolic groups of Qu facili… CuS induced localized photothermal and photodynamic therapies, and (iii) enzyme mimic response of CuS nanoc…rs contributed to the elimination of microbial pathogenesis.

1. Introduction

Wound healing requires the tissue to u…rgo suc…sive hemostasis, inflammation, proliferation a… …uration p…es [1]. The process is further complex in case of c…onic w…nds, owing … long-term infection and/or suppressed immu… respons…g in slower wound healing [2]. In particular, exudat… fluid… …d necrotic tissues present in the superficial wounds provide a fav…le environment for bacteria to initiate biofilm formation, causing chron… infections with increased risk of mortality [3]. Moreover, multidrug-r…stant (MDR) bacteria develop efflux pumps, produce hydrolytic enzymes, modify the target, block binding sites and entry ports to withstand antibiotics [4]. Current approaches to combat microbial pathogenesis include antibiotics, skin disinfectants, and hydrogels, however their clinical indications in wound healing are not fully understood [5]. Conventional broad-spectrum antibiotics are indeed very effective but play no role in wound healing [6]. In addition, continuous and rapidly growing antimicrobial resistance (AMR) has further reduced the efficacy of conventional antibiotics [7]. Clinically used skin disinfectants such as triclosan, triclocarban, and benzalkonium chloride often lead to contact dermatitis, mucous

What is Scientific Integrity?



https://students.uu.nl/sites/default/files/theunissen_integrity_masterintro_1_september.pdf

Method

- In vitro, In vivo, In silico, clinical study in human
- Who should write the method?
- **Details**
- Writing the method without copy paste!

Method and Materials: 6W

Who, What, When, Where, How, and Why: The Ingredients in the Recipe for a Successful Methods Section

Who

Who collected the specimens?

What

What reagents, methods, and instruments were used? What type of study was it? What were the inclusion and exclusion criteria for enrolling study participants? What protocol was followed? What treatments were given? What endpoints were measured? What data transformation was performed? What statistical software package was used? What was the cutoff for statistical significance? What control studies were performed? What validation experiments were performed?

When

When were specimens collected? When were the analyses performed? When was the study initiated? When was the study terminated? When were the diagnoses made?

Where

Where were the records kept? Where were the specimens analyzed? Where were the study participants enrolled? Where was the study performed?

How

How were samples collected, processed, and stored? How many replicates were performed? How was the data reported? How were the study participants selected? How were patients recruited? How was the sample size determined? How were study participants assigned to groups? How was response measured? How were endpoints measured? How were control and disease groups defined?

Why
Why was a species chosen (mice vs rats)? Why was a selected analytical method chosen? Why was a selected experiment performed? Why were experiments done in a certain order?

- **Reference in method section**

Annesley TM. Who, what, when, where, how, and why: the ingredients in the recipe for a successful Methods section. Clin Chem. 2010;56(6):897-901. doi: 10.1373/clinchem.2010.146589. PMID: 20378765.

Ethics

- In vivo study
- Clinical study
- Ethical code
- Details of ethics in experiment

Statistical Analysis

- Software and name of statistical test are not enough!
- The reason and details for selection of a statistical test.

Authorship:
Academic, social, financial.
Responsibility and accountability for published work.

Standards of reporting

- The key reporting guidelines are:
- Randomized controlled trials (RCTs): CONSORT guidelines
- Systematic reviews and meta-analyses: PRISMA guidelines and MOOSE guidelines
- Observational studies in epidemiology: STROBE guidelines
- Diagnostic accuracy studies: STARD guidelines
- Quality improvement studies: SQUIRE guidelines
- Qualitative research: SRQR or COREQ
- Economic evaluations: CHEERS
- Case reports: the CARE case report guidelines
- **Animal Research:** The ARRIVE guidelines 2.0

Why ARRIVE?

- Improving transparency in animal research
- The guidelines are relevant to any study involving live animals, from mammals to fish, as well as invertebrates, in any area of the biosciences.

The ARRIVE Essential 10

- Study design
- Sample size
- Inclusion and exclusion criteria
- Randomization
- Blinding
- Outcome measures
- Statistical methods
- Experimental animals
- Experimental procedures
- **Results**

The Recommended Set

- **Abstract**
- **Background**
- **Objectives**
- **Ethical statement**
- **Housing and husbandry**
- **Animal care and monitoring**: experimental protocols to reduce pain, Report any expected or unexpected adverse events
- **Interpretation/scientific implications**
- **Generalisability/translation**
- **Protocol registration**
- **Data access**
- **Declaration of interests**

Examples

Example 1: Ethical Issue (PubPeer)

#1 ***Pereskiopsis diguetii*** comment accepted October 2024

The mentioned ethical committee approval (IR.TBZMED) is listed in Iran National Committee for Ethics in Biomedical Research for another study, titled “Outcomes of probing surgery and monocanalicular intubation for congenital nasolacrimal duct obstruction in children older than 18 months” (https://ethics.research.ac.ir/EthicsProposalView.php?id:). Can the authors please provide the correct ethical approval?

Example 2: Details of materials

Table 1

Basic data of chemicals used: materials description, purity, and refractive index.[a]

Component	Formula	CAS	M/(kg mol^{-1})	Purity declared by the supplier (mass fraction)	Water content/ppm	
					declared	measured[b]
[Eim][Triflate]	$C_6H_9F_3N_2O_3S$	501693–46-5	0.24621	> 0.98	714	742
[Epy][Triflate]	$C_8H_{10}F_3NO_3S$	3878–80-6	0.25732	> 0.99	929	870
~~water~~	H_2O	7732–18-5	0.01802	~~de ionized~~	–	–
ethanol	C_2H_6O	64–17-5	0.04607	> 0.999	> 1000	225

[a] Experimental pressure is 100 kPa and standard uncertainties, u, are: $u(n_D) = 0.0002$, $u(t) = 0.05$ °C and $u(P) = 3$ kPa.

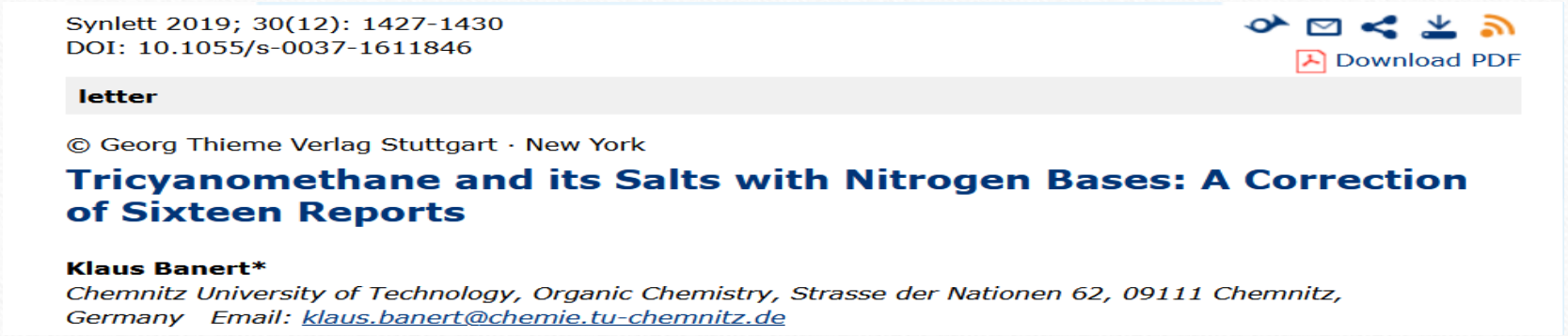
[b] Estimated by Karl Fischer titration for the supplied components.

Example 3: Details of materials and instrument

- **Examples:**
- Method: We performed temperature of experiment at **±0.1 K**: Obtained data at **298.15 K**.
- HCl (63%)!
- We determined the drug concentration by ELISA kit (?)!
- Another personal example

Example 4

- Tricyanomethane as starting materials for synthesis (16 reports) and its salt forms were reported.



Synlett 2019; 30(12): 1427-1430
DOI: 10.1055/s-0037-1611846

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Tricyanomethane and its Salts with Nitrogen Bases: A Correction of Sixteen Reports

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- The authors stated that they did not report the synthesis of tricyanomethane in the published paper as they purchased this compound from a commercial center and used it in the synthesis.
- Retracted!

Conclusion: Important recommendations

- Who should write the method?
- **Details**
- Writing the method without copy paste!