Biocompatibility of Restorative Dental Materials and Related Researches

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Outlines

- *Introduction*
- *In vitro tests*
  - *Cytotoxicity Tests*
  - *Tests for Cell Metabolism or Cell Function*
  - *Tests that Use Barriers (Indirect tests)*
  - *Other Assays for Cell Function*
  - *Mutagenesis Assays*
- *Animal Tests*
  - *The mucous membrane irritation tests*
  - *Implantation tests*
- *Usage Tests*
  - *Dental Pulp Irritation Tests*
  - *Dental Implants into Bone*
  - *Mucosa and Gingival Usage Tests*
- *Correlation among In Vitro, Animal, and Usage Tests*
- *Using In Vitro, Animal, and Usage Tests together*
- *Related Researches for Restorative Dental Materials*

Properties of Dental Materials

- Physical & Mechanical properties
  - Strength (Compressive, flexural, Toughness, Bond)
  - Hardness
  - Thermal activity (Conductivity, Diffusivity)
  - Water sorption and solubility
- Chemical Properties
  - Corrosion
  - Stability
- Biological properties ???

Biocompatibility

Non-properties:

non-toxic, non-immunogenic, non-thrombogenic,

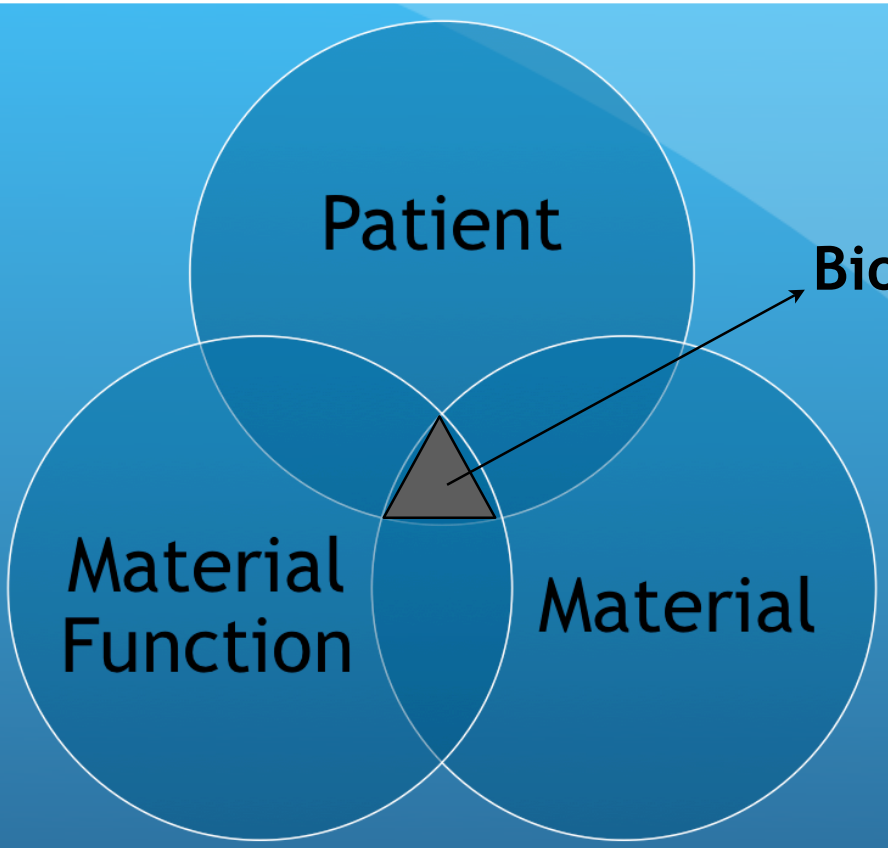
non-carcinogenic, etc.

Function of materials ???

Truly inert property ???

" ability of a material to perform with an appropriate host response in a specific situation."

Williams DF., 1987



"Practitioners should understand that there are no inert materials. When material is placed into living tissue, interaction with the complex biologic systems around it occur, and those interactions result in some sort of biologic response.

Wataha J.C., 2001

The core `dogma' of biocompatibility

Wataha JC., 2012

The interactions at material-tissue interface occur for both.

The material-tissue interface is dynamic.

The reactions at the material-tissue interface are the function of the tissue where the interface is created.

Materials we used do not belong there, all biomaterials are always foreign bodies.

It is possible to customize interactions at the materials-tissue interface.

Biocompatibility

"ability of a biomaterial to perform its desired function with respect to a medical therapy, without eliciting any undesirable local or systemic effects in the recipient or beneficiary of that therapy, but generating the most appropriate beneficial cellular or tissue response to that specific situation, and optimizing the clinically relevant performance of that therapy"

Williams DF., 2008

Introduction

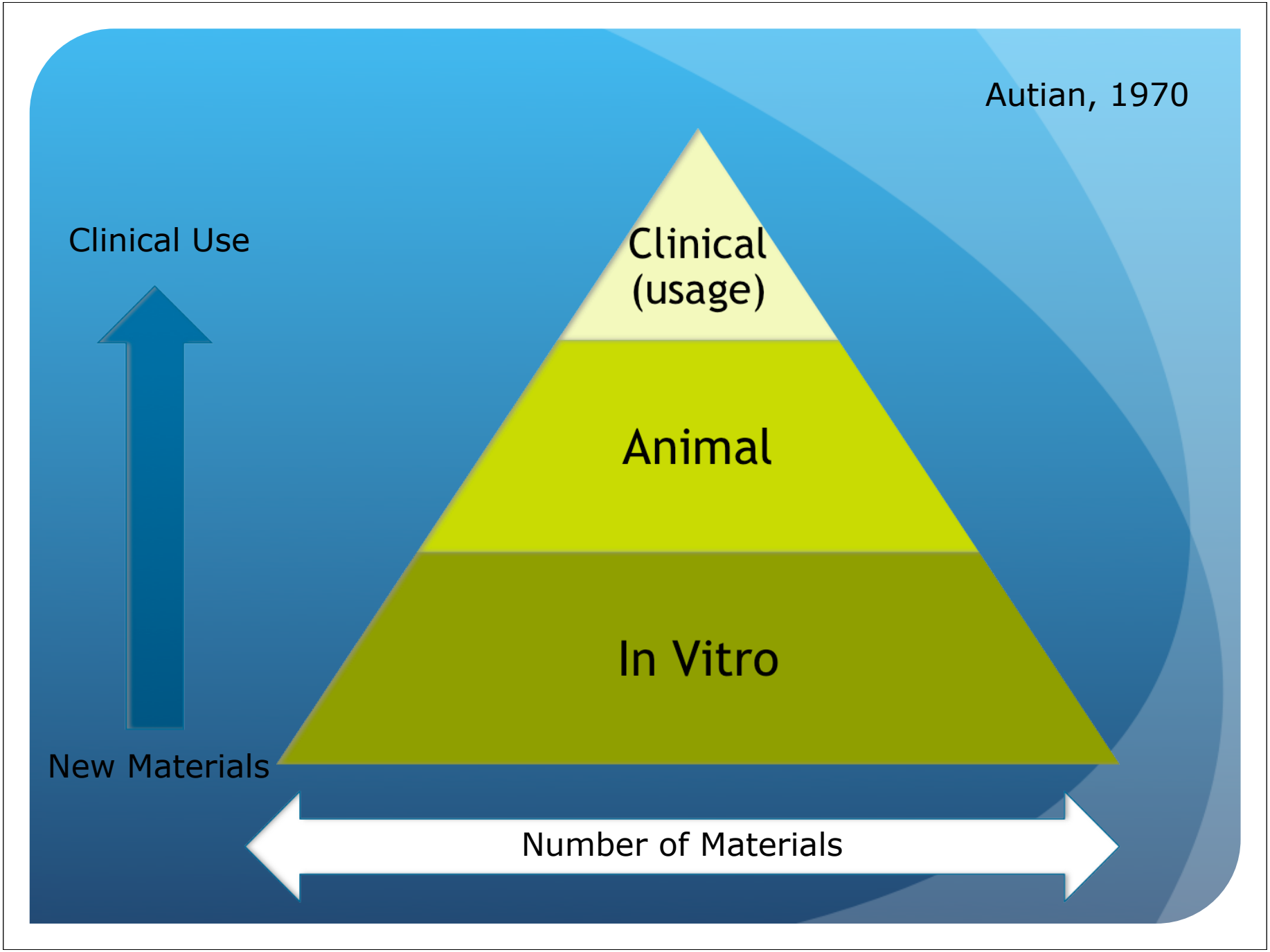
- Measuring the biocompatibility of a material is not simple,
- The methods of measurement are evolving rapidly as more is known about the interactions between dental materials and oral tissues and as technologies for testing improve.

Introduction

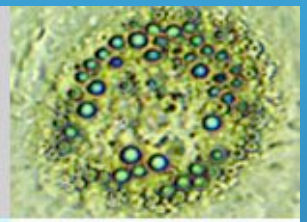
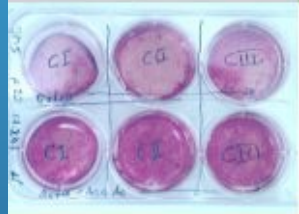
Historically, new materials were simply tried in humans to see if they were biocompatible. However, this practice has not been acceptable for many years, and current materials must be extensively screened for biocompatibility before they are ever used in humans.

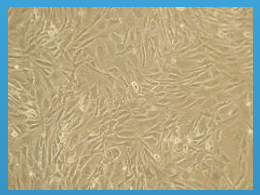
Introduction

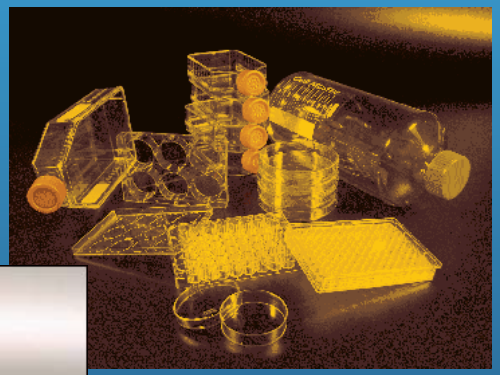
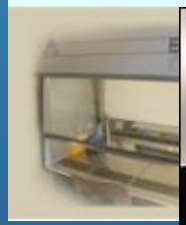
- Several varieties of tests are currently used to try to ensure that new materials are biologically acceptable.
- These tests are classified as in vitro, animal, and usage tests.
- These three testing types include the clinical trial, which is really a special case of a usage test in humans.


Autian, 1970
Clinical Use
Clinical
(usage)
Animal
In Vitro
New Materials
Number of Materials

In vitro (Latin: in glass) tests




In Vitro tests

- 1926: Cell-culture technique
- 1968: Kawahara reported ‘Cytotoxicity test’ including dental materials.
- 1972: Leirsker & Helgeland reported the use of L-929 cells to assess biocompatibility of amalgam, resin, silicate cement and gold base alloy.
- 1973: Spangberg reported the 1st quantitative measure of biological response in vitro using ^{51}Cr assay.
- 1977: Agar overlay test.
- In vitro purpose to simulate the in vivo conditions in any aspect of cell function or metabolism, including gene expression, signaling activation, protein expression, oxidative stress, etc.

In vitro tests

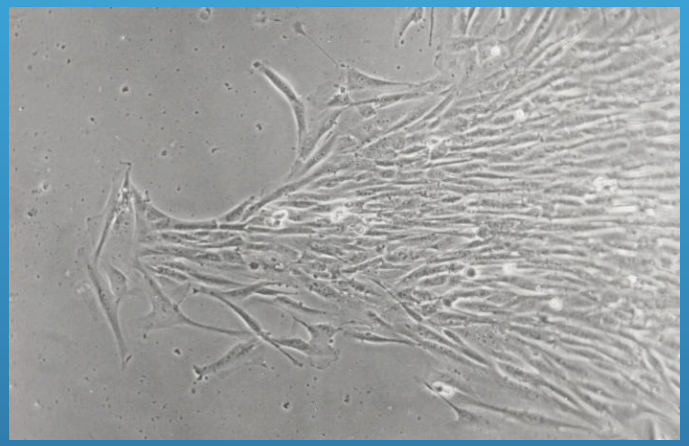
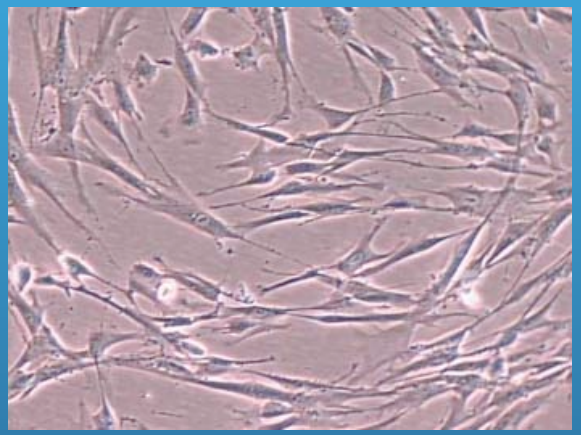
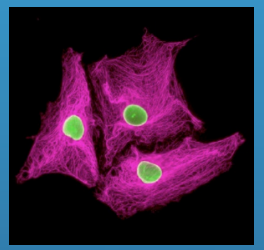
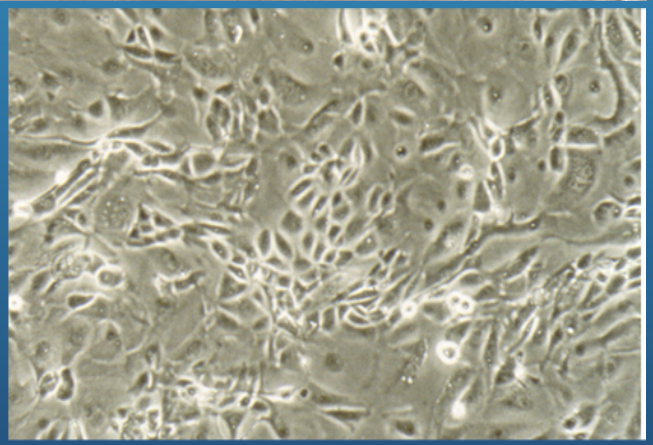
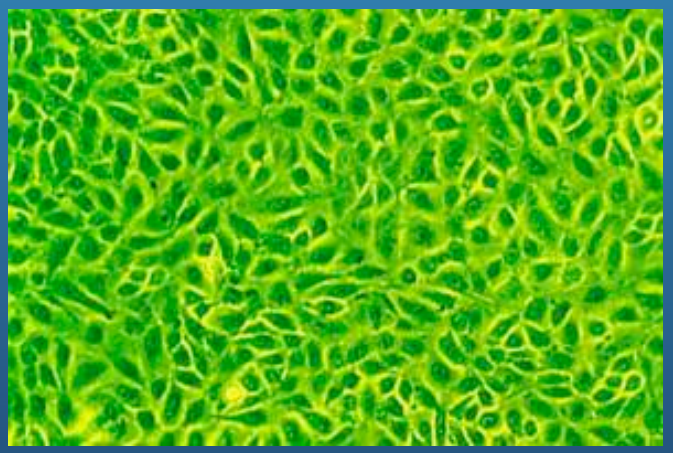
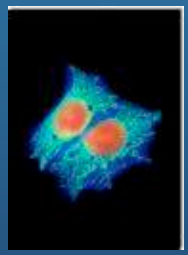
Strengths:

- The ability to control the environment of the cells and their interface with materials.
- The ability to measure cell response in detail and with precision.
- In vitro tests are faster, less expensive, more producible and more scalable.

Weakness:

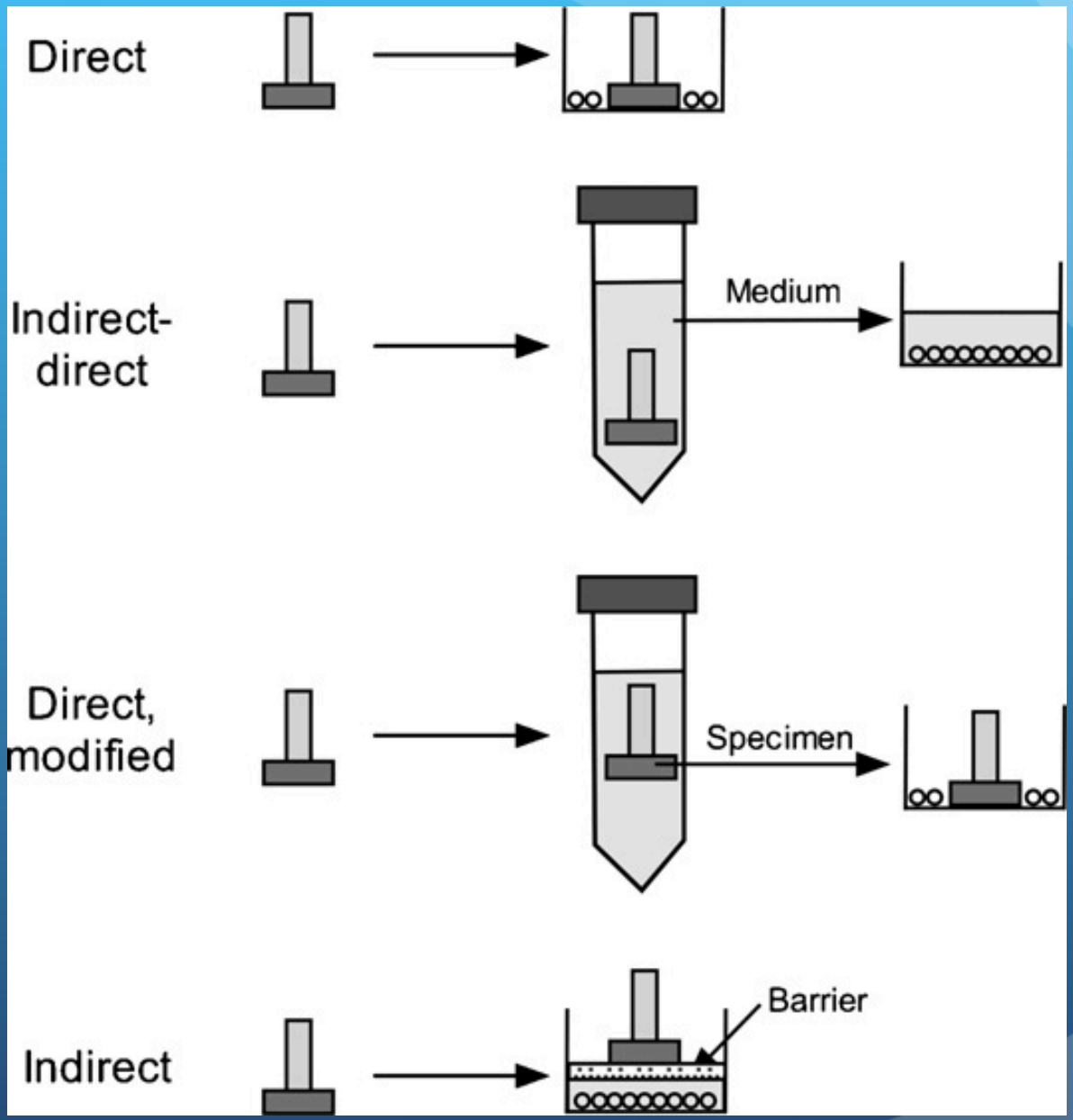
- The lack of relevance to the Clinical use.

Cell Culture: Human

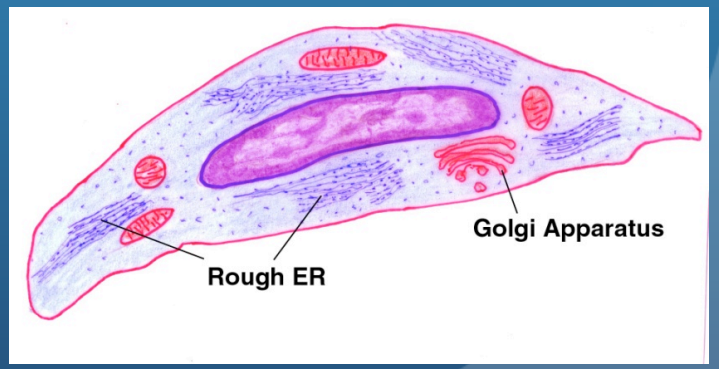
In vitro tests

- Two types of cell can be used for in vitro assays.
  - *Primary cells* are cells taken directly from an animal into culture. These cells will grow for only a limited time in culture but may retain many of the characteristics of cells in vivo.
  - *Continuous cells* are primary cells that have been transformed to allow them to grow more or less indefinitely in culture. Because of their transformation, these cells may not retain all in vivo characteristics, but they consistently exhibit any features that they do retain.


Direct
Indirect-
direct
Medium
Direct,
modified
Specimen
Indirect
Barrier

In vitro tests

- *Cytotoxicity Tests*
- *Tests for Cell Metabolism or Cell Function*
- *Tests that Use Barriers (Indirect tests)*
- *Other Assays for Cell Function*
- *Mutagenesis Assays*

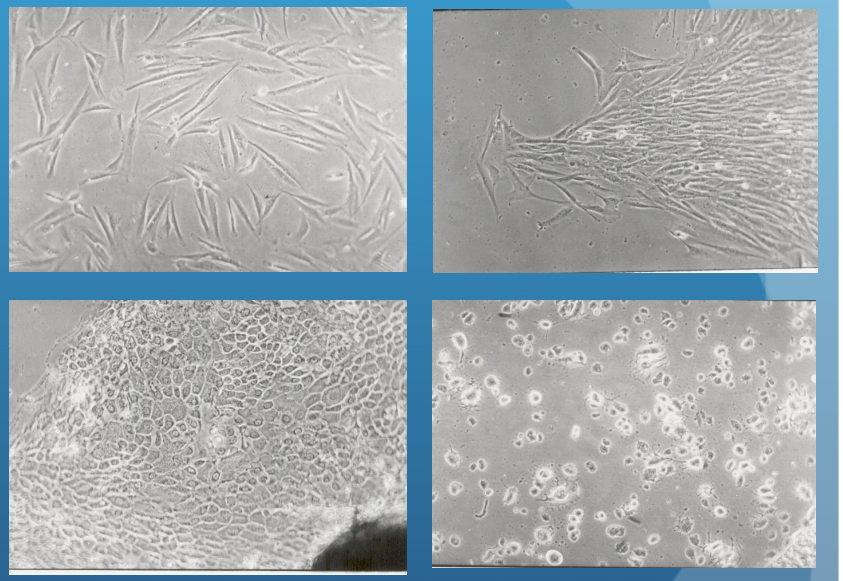


Cytotoxicity Tests

- Cytotoxicity tests assess the cytotoxicity of a material by measuring cell number or growth after exposure to a material.
- Cells are plated in a well of a cell-culture dish where they attach.
- The material is then placed in the test system.

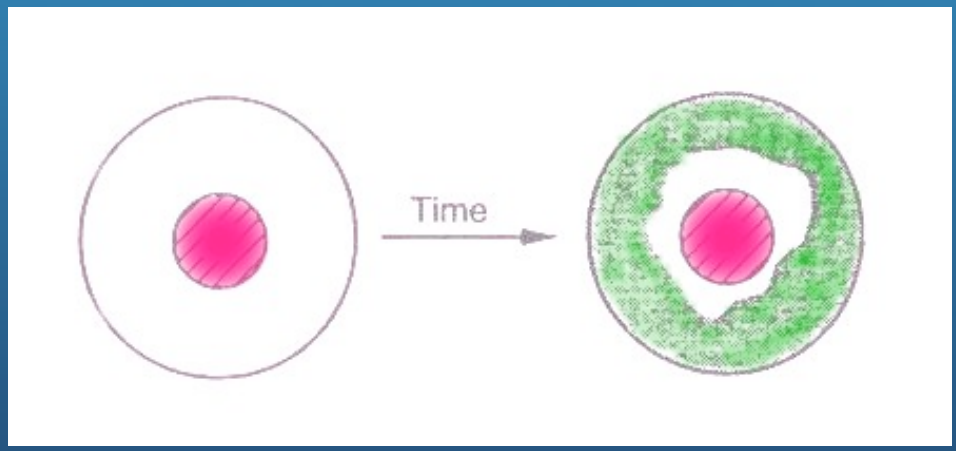
Cytotoxicity Tests

- If the material is not cytotoxic, the cells will remain attached to the well and will proliferate with time.
- If the material is cytotoxic, the cells may stop growing, exhibit cytopathic features, or detach from the well.



Cytotoxicity Tests

- If the material is a solid, then the density (number of cells per unit area) of cells may be assessed at different distances from the material, and a zone of inhibited cell growth may be described.

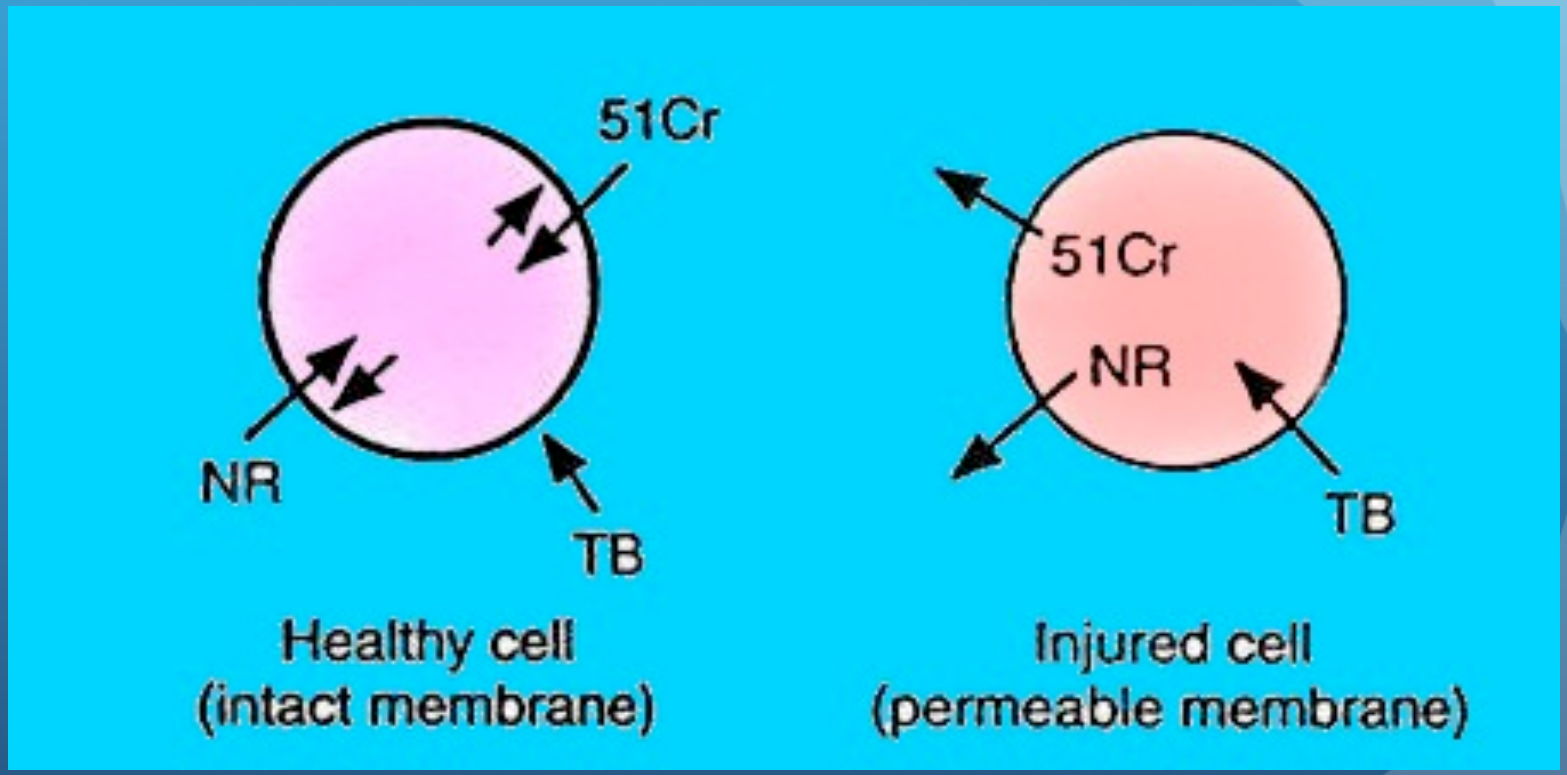


Cell Culture: Ring of Inhibition

Cytotoxicity Tests

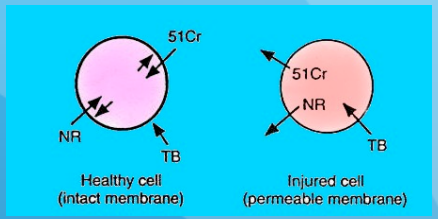
- Another group of tests is used to measure cytotoxicity by a change in membrane permeability.
- *Membrane permeability* is the ease with which a dye can pass through a cell membrane.
- This test is used on the basis that a loss in membrane permeability is equivalent to or very nearly equivalent to cell death.

a change in membrane permeability



NR = Nutral Red TB= Trypan Blue

Cytotoxicity Tests



- There are two basic types of dyes used.
  - Vital dyes are actively transported into viable cells, where they are retained unless cytotoxic effects increase the permeability of the membrane. It is important to establish that the dye itself does not exhibit cytotoxicity during the time frame of the test.
  - Nonvital dyes are not actively transported, and are only taken up if membrane permeability has been compromised by cytotoxicity.

Cytotoxicity Tests

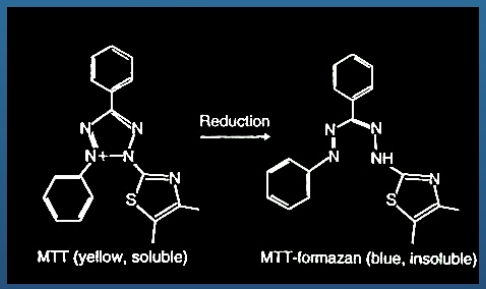
- The advantages of the membrane permeability test is that it identifies cells that are alive (or dead) under the microscope.
- This feature is important because it is possible for cells to be physically present, but dead (when materials fix the cells).

Tests for Cell Metabolism or Cell Function

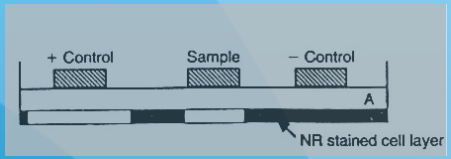
- Some in vitro tests for biocompatibility use the biosynthetic or enzymatic activity of cells to assess cytotoxic response.
- Tests that measure deoxyribonucleic acid (DNA) synthesis or protein synthesis are common examples of this type of tests.

Tests for Cell Metabolism or Cell Function

- A commonly used enzymatic test for cytotoxicity is the MTT test. This test measures the activity of cellular dehydrogenases, which convert a chemical called MTT, via several cellular reducing agents, to a blue, insoluble formazan compound

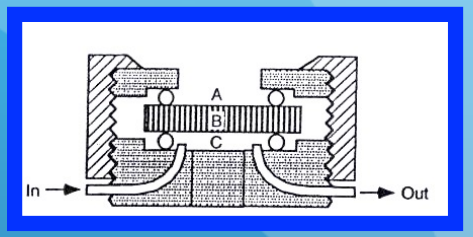


Tests that Use Barriers (Indirect tests)



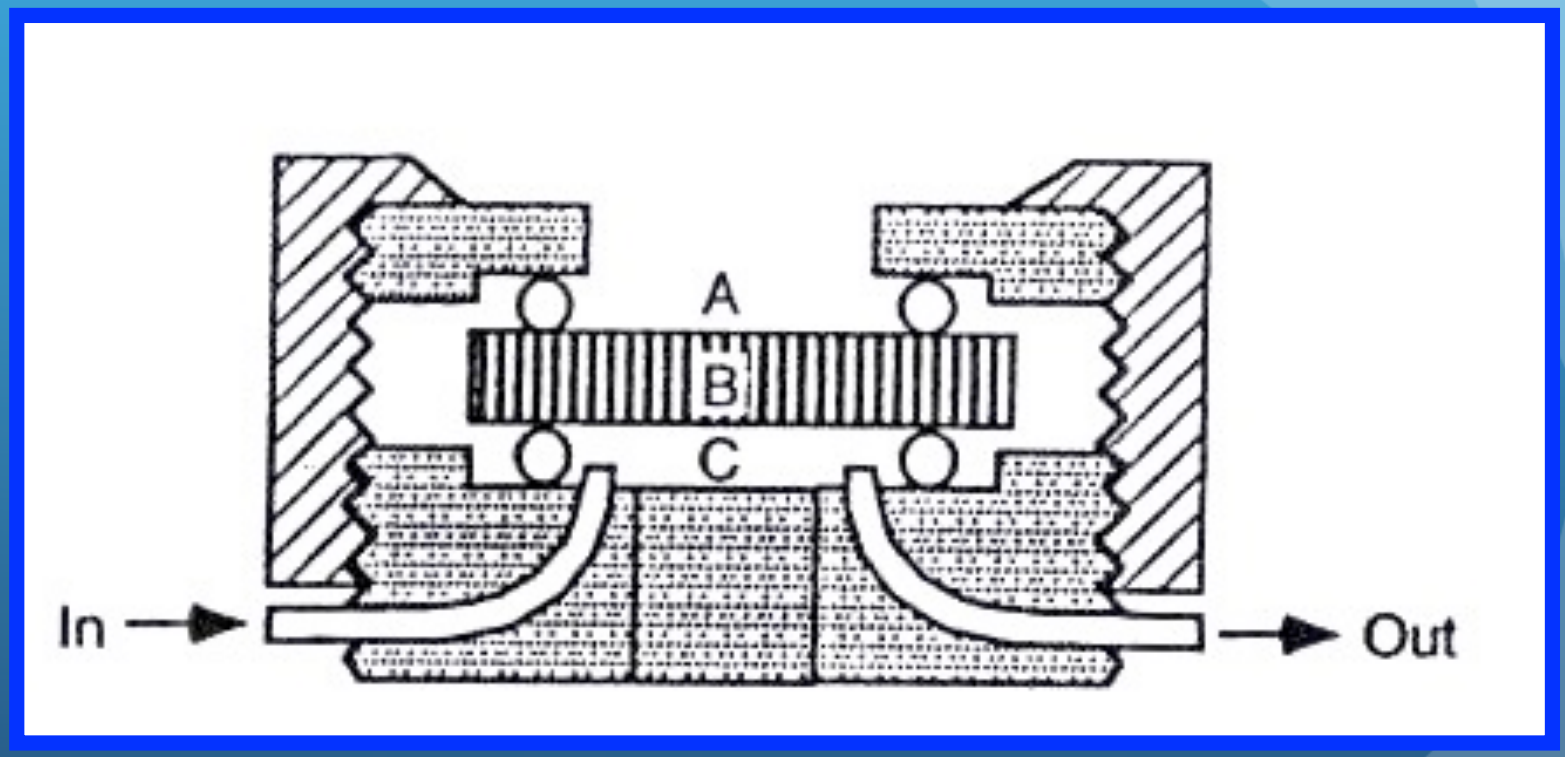
- Thus several in vitro barrier tests have been developed to mimic in vivo conditions.
- One such test is the *agar overlay method* in which a monolayer of cultured cells is established before adding 1% agar or agarose (low melting temperature) plus a vital stain, such as neutral red, to fresh culture media.

Tests that Use Barriers (Indirect tests)

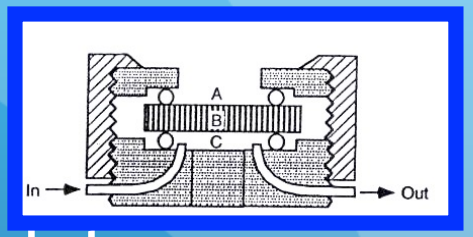


- *Dentin Barrier tests* have shown improved correlation with the cytotoxicity of dental materials in usage tests in teeth, and are gradually being developed for screening purposes
- A number of studies have shown that dentin forms a barrier through which toxic materials must diffuse to reach pulpal tissue.
- Pulpal reaction to zinc oxide-eugenol is relatively mild as compared with the more severe reactions to the same material in direct contact with cells in vitro assays and tissue in implantation tests.

Dentin Barrier tests



Tests that Use Barriers (Indirect tests)



- The thickness of the dentin correlates directly with the protection offered to the pulp.
- Thus assays have been developed that incorporate dentin disks between the test sample and the cell assay system.
- The use of dentin disks offers the added advantage of directional diffusion between the restorative material and the culture medium.

Other Assays for Cell Function

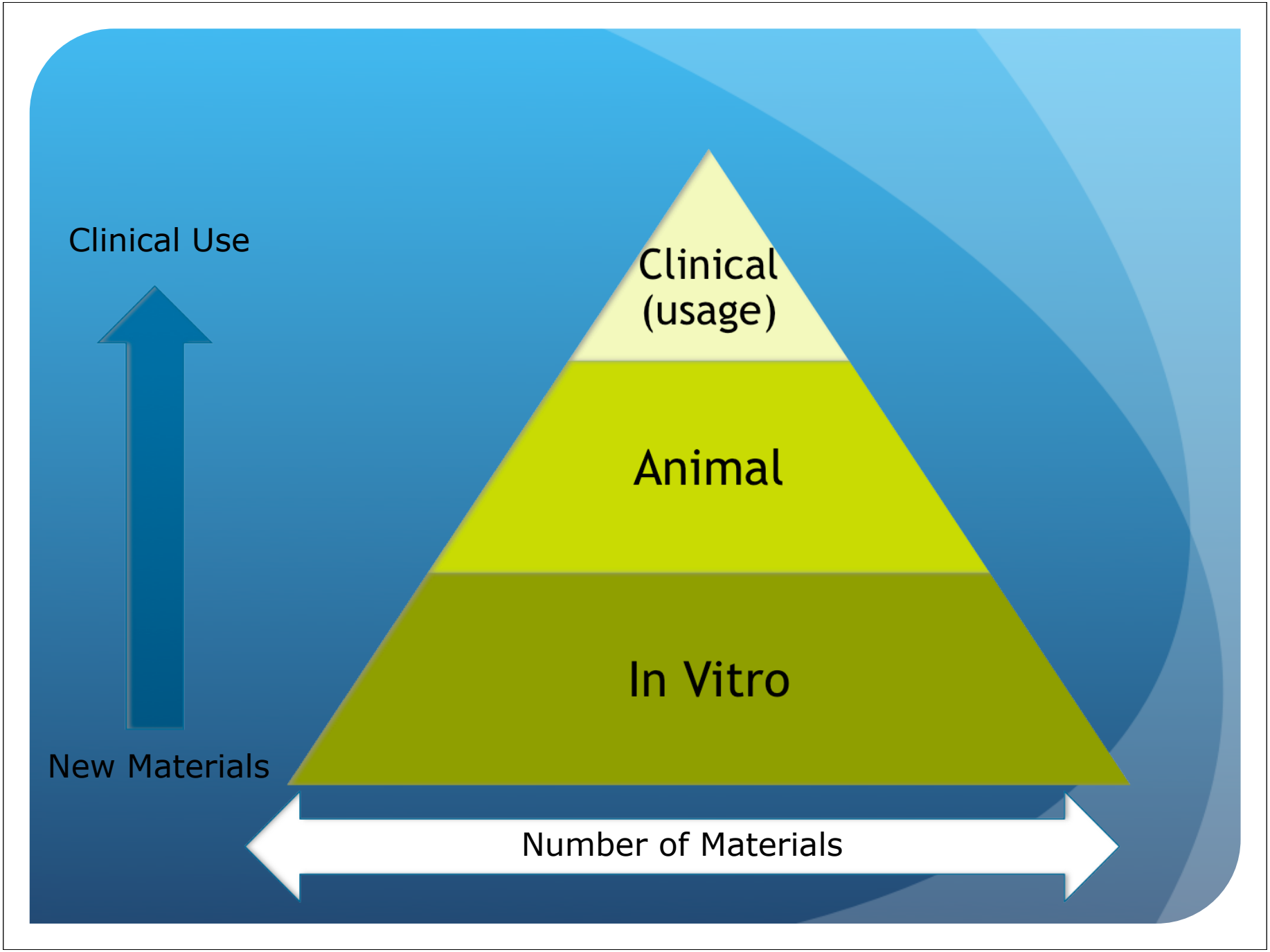
- In vitro assays to measure **immune function** or other tissue reactions have also been used.
- These assays measure **cytokine production** by lymphocytes and macrophages, lymphocyte proliferation, or T-cell resetting to sheep red blood cells.
- Other tests measure the ability of a material to alter the cell cycle or activate compliment.

Mutagenesis Assays

- Mutagenesis assays assess the effect of materials on a cell's genetic materials.
- Genotoxic mutagens directly alter the DNA of the cell through various types of mutations. Each chemical may be associated with a specific type DNA mutation.
- Genotoxic chemicals may be mutagens in their native states, or may require activation or biotransformation to be mutagens, in which case they are called *Promutagens*.

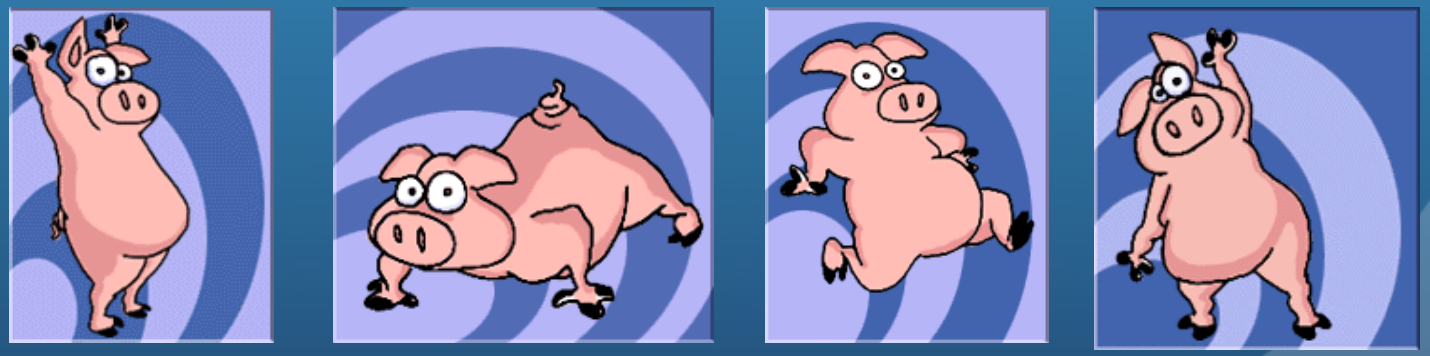
Mutagenesis Assays

- Epigenetic mutagens do not alter the DNA themselves, but support tumor growth by altering the cell's biochemistry, altering the immune system, acting as hormones, or other mechanisms.
- *Carcinogenesis* is the ability to cause cancer in vivo.
- Mutagens may or may not be carcinogens, and carcinogens may or may not be mutagens.
- Thus the quantification and relevance of tests that attempt to measure mutagenesis and carcinogenesis are extremely complex.


Clinical Use
New Materials
Clinical
(usage)
Animal
In Vitro
Number of Materials

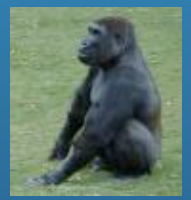
Animal Tests

- *The mucous membrane irritation tests*
- *Implantation tests*



Animal Tests

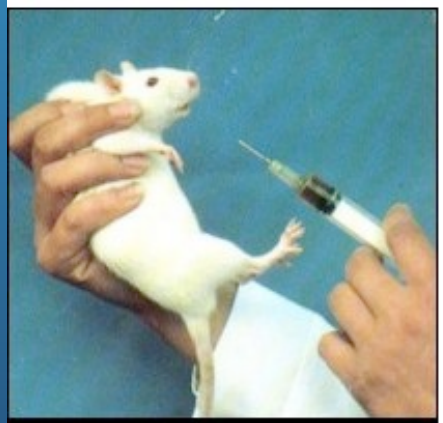
- Animal tests for biocompatibility are usually used in mammals such as mice, rats, hamsters, or, guinea pigs, although many types of animals have been used.

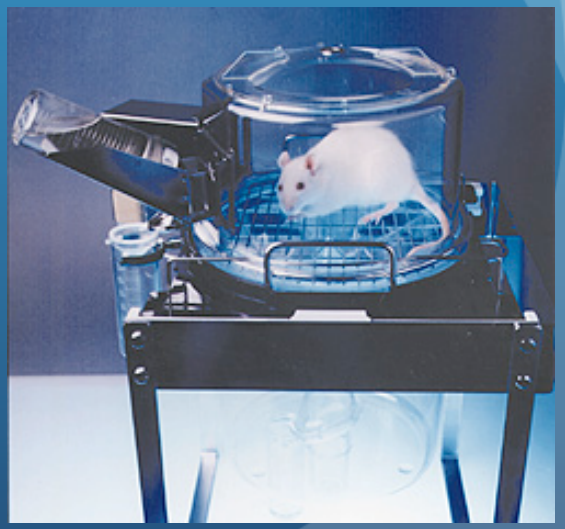


Animal Tests

- Animal tests are distinct from usage tests (which are also often done in animals) in that the material is not placed in the animal with regard to its final use.
- The use of an animal allows many complex interactions between the material and a functioning, complete biological system to occur.
- For example, an immune response may occur or complement may be activated in an animal system in a way that would be difficult to mimic in a cell-culture system.

Animal Tests


Rat used in acute toxicity testing.



Animal Tests

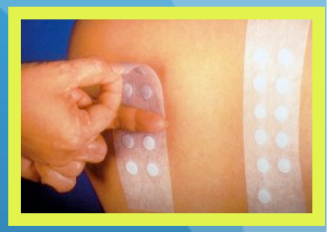
- The biological responses in animal tests are more comprehensive and may be more relevant than in vitro tests, and these are the major advantages of these tests.
- The main disadvantages of animal tests are that they can be difficult to interpret and control, are expensive, may be time consuming, and often involve significant ethical concerns and paperwork.
- The relevance of the test to the in vivo use of a material can be quite unclear, especially in estimating the appropriateness of an animal species to represent a human.
- A variety of animal tests have been used to assess biocompatibility

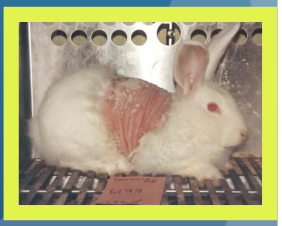
The mucous membrane irritation tests

- The mucous membrane irritation test determines if a material causes inflammation to mucous membranes or abraded skin.
- This test is conducted by placing the test materials and positive and negative controls into contact with hamster cheek-pouch tissue or rabbit oral tissue.
- After several weeks of contact, the controls and test sites are examined, and the gross tissue reactions in the living animals are recorded and photographed in color.
- The animals are then sacrificed, and biopsy specimens are prepared for histological evaluation of inflammatory changes.

The skin sensitization tests

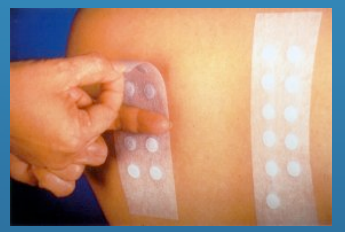
- In the skin sensitization test in guinea pigs, the materials are injected intradermally to test for development of skin hypersensitivity reactions.
- This injection is followed by secondary treatment with adhesive patches containing the test substance.
- If hypersensitivity developed from the initial injection, the patch will elicit an inflammatory response.
- The skin-patch test can result in a spectrum from no reaction to intense redness and swelling.
- The degree of reaction in the patch test and the percentage of animals that show a reaction are the bases for estimating the allergenicity of the material.





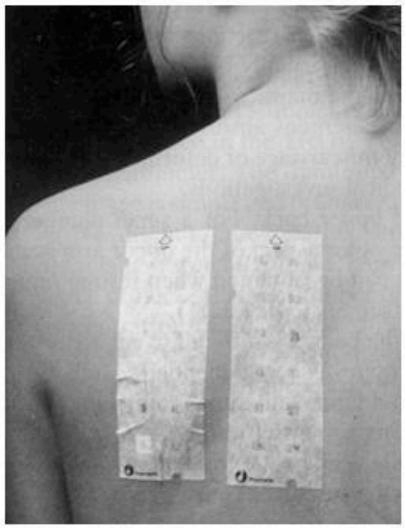
The skin sensitization tests

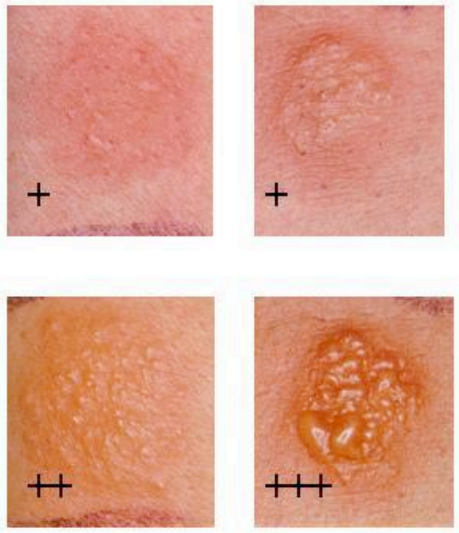
The skin sensitization tests



Sensitization: Diagnostic test

Patch Test





Figures modified from J. E. Wahlberg, Patch Testing, Texbook of Contact Dermatitis

Implantation tests

- To evaluate materials that will contact subcutaneous tissue or bone
- The location of the implant site is **determined by the use of the material**, and may include connective tissue, bone, or muscle.
- Although amalgams and alloys are tested because the margins of the restorative materials contact the gingival, most subcutaneous tests are used for materials that will directly contact soft tissue during implantation, endodontic, or periodontal treatment.
- Short-term implantation is studied by aseptically placing the compounds in small, open-ended, polyethylene tubes into the tissue.
- The test samples and control are placed at separate sites, and allowed to remain for 1 to 11 weeks.

Implantation tests

- The tissue response can be evaluated by normal histological, histochemical, or immunohistochemical methods.
- Implantation tests of longer duration, for identification of either chronic inflammation or tumor formation, are performed in a manner similar to that of short-term tests except the materials remain in place for 1 to 2 years before examination.

Animal tests

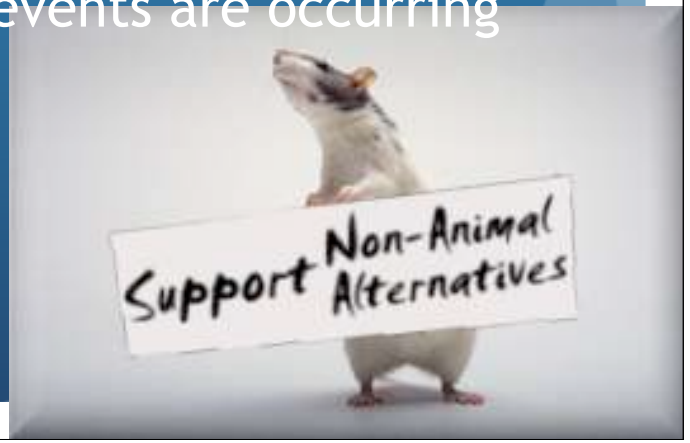
Strengths :

- The **ability to assess the biological response that cannot modeled by in vitro test**, including blood interaction, wound healing, infection, hypersensitivity response, carcinogenesis and chronic inflammation, etc.
- Generally **less expensive** than human clinical trials.
- The ability to **completed more quickly and can be controlled to a grater degree**.
- Animals may be test in **many stages of life** (embryo, children) in manner that is not possible in humans. .

Animal tests

Weakness:

- Due to the species differences, the congruity of animal response to human response cannot be assumed, and may be, at worst, misleading.
- Limitation of an animal tests to mimic the human-material interface, for example occlusal force and food, etc.
- Interpretation of response is complex in animal tests because many overlapping complex events are occurring simultaneously.
- Ethical and cost considerations

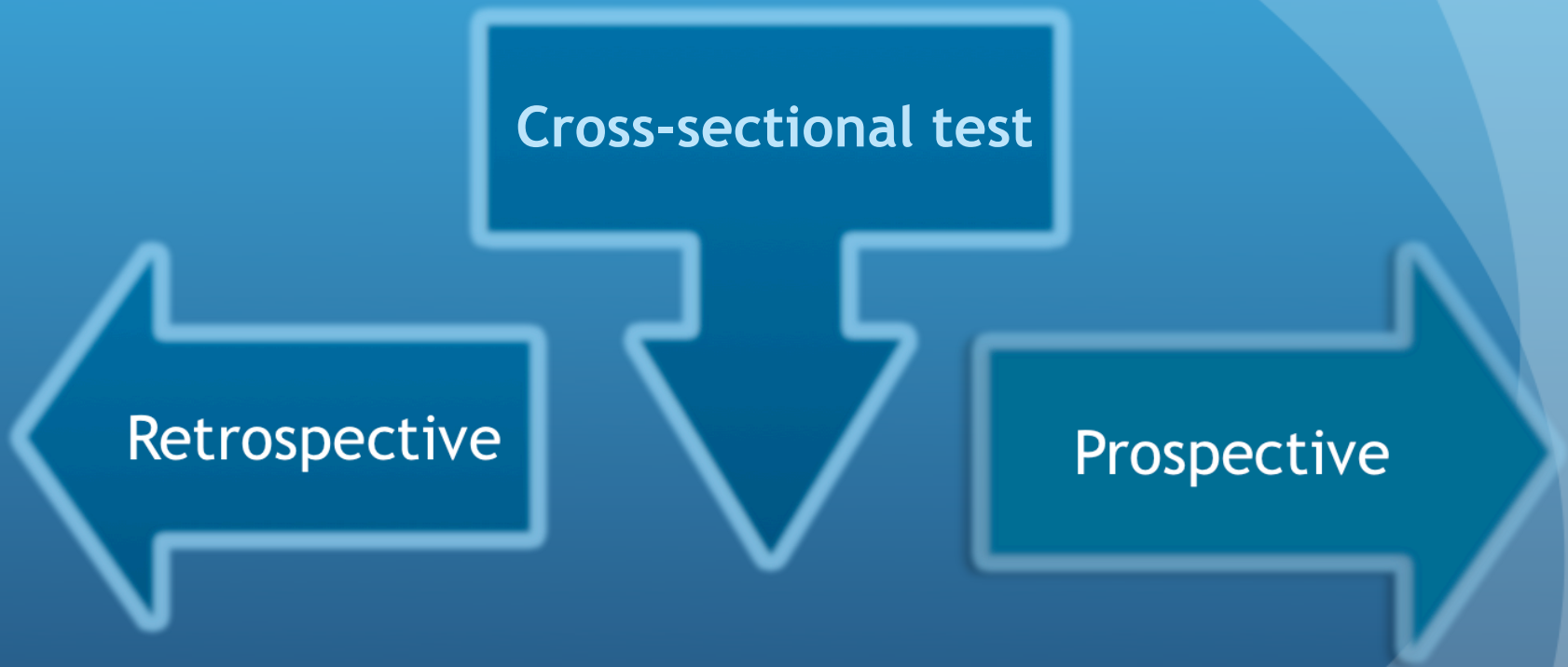


Clinical Use

New Materia

Number of Materials

Clinical tests (in human)



Retrospective test

"Reviewing of the patient records after the fact to assess material performance."

Strengths:

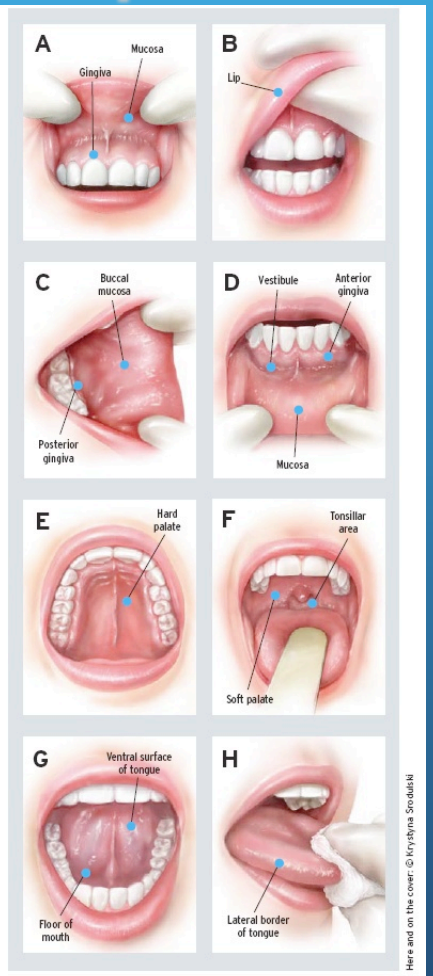
- Simplest and least expensive
- Do not require direct patient examination

Weakness:

- Heavily depend on the quality of information that recorded.
- The risk of selection bias, due to the data quality and past practitioners.

Cross-sectional test

"A patient cohort examined at one point in time."



Here and on the cover: © Krystyna Szoduski

FIGURE 1. A brief screen for oral cancer includes this eight-step examination of the inside of the mouth.

Strengths:

- Ability to define exclusion and inclusion criteria.
- Collect specific data in standardized condition.

Weakness

- Lack of control of how material was used.
- The variables that may have been important but were unrecorded.
- Skills and limitation of examiner.

Prospective/Longitudinal test

Controlled clinical trials/Randomized control trials

Strengths:

- Assure blinding, randomization and placebo.
- The most reliable and interpretable information

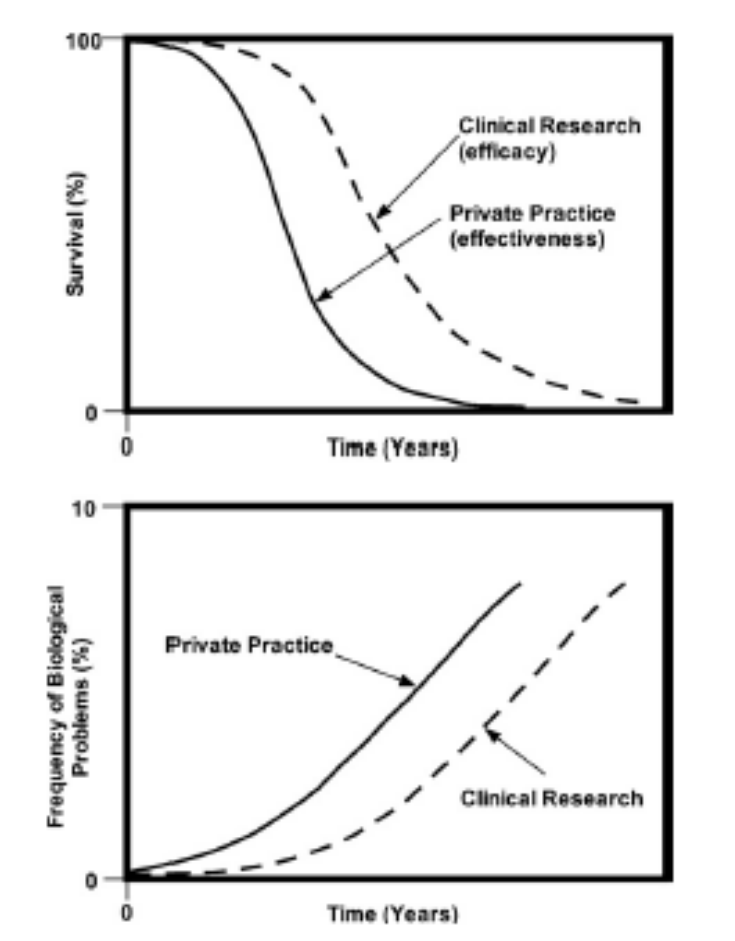
Weakness:

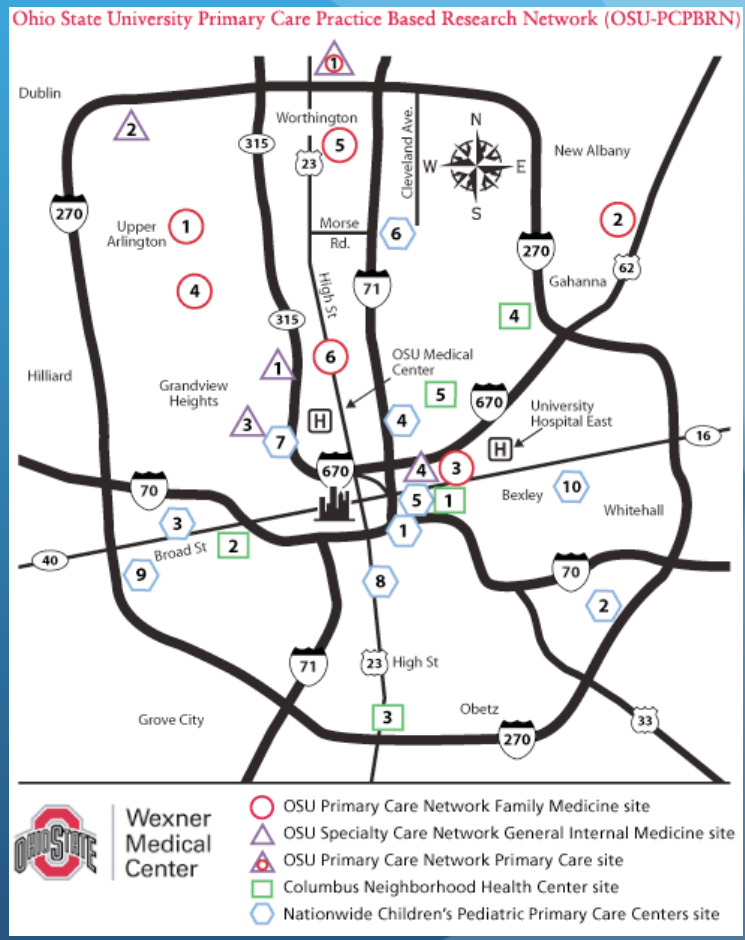
- Skill of the operator may be cannot represent the ability of average practitioner.
- The disease stage treated may not be relevant to clinical practice.
- Expensive and time consuming

Simple clinical trials & Practice-base research networks

- Faster and less expensive
- Simple clinical trials offer a clinical view of material performance, but without stringency for controls, blindness or randomized designs.
- The practitioner in the network are calibrated for assessing outcomes and trained to adhere to similar protocols.
- There are countries that created national database for practitioners to report adverse events post-market introduction, with the goal of using a very large sample size.

Clinical research vs Practitioner





Bayne S.G, 2007

FDI system for clinical evaluation., 2010

- Tooth vitality and hypersensitivity
- Changes in the periodontal or adjacent mucosal tissues
- Effect to general health

Advantages and Disadvantages of Biocompatibility Tests

From Wataha JC; Biocompatibility of Dental Materials; Chapter 5 from Craig RG & Powers JM, Restorative Dental Materials, 11th Edition, 2002 Mosby, Inc. Page 135

test	Advantages	Disadvantages
In vitro tests	Quick to perform Least expensive Can be standardized Large-scale screening Good experimental control Excellence for mechanisms of interactions	Relevance to in vivo is questionable
In vivo tests	Allows complex systemic interactions Response more comprehensive than in vitro tests More relevant than in vitro tests?	Relevance to use of material questionable Expensive Time consuming Legal/ethical concerns Difficult to control Difficult to interpret and quantify
Usage tests	Relevance to use of material is assured	Very expensive Very time consuming Major legal/ethical issues Con be difficult to control Difficult to interpret and quantify

Standard of biocompatibility

- American National Standard Institute(ANSI) via ADA
- American Society of Testing and Materials(ASTM)
- The Committee on European Normalization(CEN)
- The International Organization of Standardization(ISO)
- Nordic institute of Dental Materials(NIOM)
- The European Union(EN)

Organization	Standard	Year	Title
ANSI/ADA	41	2005	Recommended standard practices for biological evaluation of dental materials
ASTM-Int			
	F1027-86	2007	Standard practice for assessment of tissue and cell compatibility of orofacial prosthetic materials and devices
	F1538-03	2009	Standard specification for glass and glass ceramic biomaterials for implantation
	F748-06	2010	Standard practice for selecting generic biological test methods for materials and devices
	F1609-08	2008	Standard specification for calcium phosphate coatings for implantable materials
	F1876-98	2003	Standard specification for polyetherketoneetherketonekone (PEKEKK) resins for surgical implant applications
	F2026-10	2010	Standard specification for polyetheretherketone (PEEK) polymers for surgical applications
	F1441-30	2009	Standard specification for soft-tissue expander devices
	F2211-04	2004	Standard classification for tissue engineered medical products (TEMPs)
	F523-99	2006	Standard guide for pre-clinical *in vivo* evaluation in critical size segmental bone defects
CEN	EN 1640-1642	2009	Medical devices for dentistry (4 parts)
ISO	10993		
	10993-1	2009	Part 1: Evaluation and testing
	10993-2	2006	Part 2: Animal welfare requirements
	10993-3	2003	Part 3: Tests for genotoxicity, carcinogenicity and reproductive toxicity
	10993-4	2002/2006	Part 4: Selection of tests for interactions with blood
	10993-5	2009	Part 5: Tests for *in vitro* cytotoxicity
	10993-6	2007	Part 6: Tests for local effects after implantation
	10993-7	2008	Part 7: Ethylene oxide sterilization residuals
	10992-8	2001	Part 8: Selection of reference materials
	10993-9	1999	Part 9: Framework for identification and quantification of potential degradation products
	10993-10	2010	Part 10: Tests for irritation and delayed-type hypersensitivity
	10993-11	2006	Part 11: Tests for systemic toxicity
	10993-12	2007	Part 12: Sample preparation and reference materials
	10993-13	1998	Part 13: Identification and quantification of degradation products from polymeric medical devices
	10993-14	2001	Part 14: Identification and quantification of degradation products from ceramics
	10993-15	2000	Part 15: Identification and quantification of degradation products from metals and alloys
	10993-16	1997	Part 16: Toxicokinetic study design for degradation products and leachables
	10993-17	2002	Part 17: Establishment of allowable limits for leachable substances
	10993-18	2005	Part 18: Chemical characterization of materials
	10993-19	2006	Part 19: Physico-chemical, morphological and topographical characterization of materials
	10993-20	2006	Part 20: Principles and methods for immunotoxicology testing of medical devices
	7405	2008	Evaluation of biocompatibility of medical devices used in dentistry
	14155	2011	Clinical investigation of medial devices for human subjects—good clinical practice.
	14971	2007	Application of risk management to medical devices

ANSI/ADA, American National Standards Institute/American Dental Association (http://www.ansi.org or http://www.ada.org); ASTM-Int, American Society for Testing and Materials International (http://www.astm.org); CEN, Comité Européen de Normalisation (http://www.cenorm.be); ISO, International Organization for Standardization (http://www.iso.org). *Other Organizations*: EN, European Union (http://europa.eu); FDA, U.S. Food and Drug Administration (http://www.fda.gov/medicaldevices); GHTF, Global Harmonization Task Force (http://www.ghtf.org); NIOM, Nordic Institute for Dental Materials (http://www.niom.no).

U.S. FDA 510(k) or grandfather clause

- ★ Dose the new device have the same intended use as equivalent device ?
- ★ Does the new device have technological characteristics that rinse new types of safety or effectiveness concerns ?
- ★ Dose performance data demonstrate equivalence ?

ONLY FOR CLASS I OR CLASS II DEVICES

FDI classification of common dental devices

Material	Section	Class	Comment
Facebow	872.3220	I	
Dental articulator	872.3150	I	
Dental cements	872.3275	I	- Zinc oxide eugenol, temporary
		II	- Non-zinc oxide eugenol, permanent
Intraoral dental wax	872.6809	I	
Alloy, base metal	872.3710	II	- Special controls in guidance document 08/23/2004[b]
Alloy, noble metal	872.3060	II	- Special controls in guidance document 08/23/2004
Amalgam	872.3070	II	- Special controls in guidance document 07/28/2008
Calcium hydroxide liner	872.3250	II	
Endosseous implant	872.3640	II	- Root forms, special controls in guidance document 02/12/2004
		III	- Blade forms
Impression materials	872.3660	II	- Special controls in guidance document 04/22/2003
Pit and fissure sealants	872.3265	II	
Porcelain powder for clinical use	872.6660	II	
Pre-formed plastic dental teeth	872.3590	II	
Resin bonding agent	872.3200	II	
Root canal filling resin	872.3820	II	- Formulations without chloroform
		III	- Formulations with chloroform
Tooth shade resin material	872.3690	II	

[a] FDA website: http://www.fda.gov/MedicalDevices/default.htm; this list is not complete; see website for a complete list.
[b] Guidance documents are issued periodically and provide information on conditions that might limit or modify the overall classification of the device; available at FDA website.

Future of biocompatibility testing

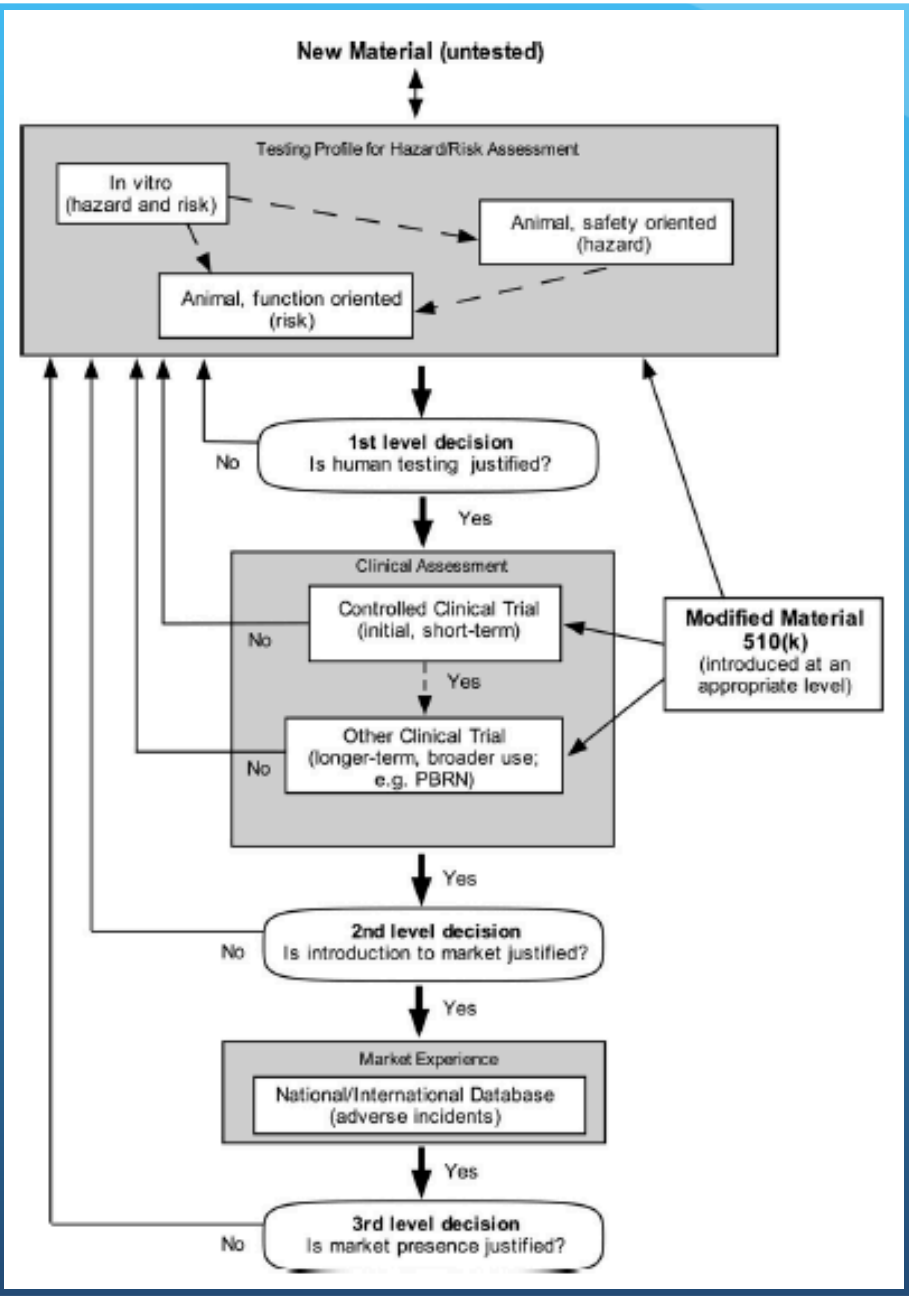
Hazard: The potential for the material to cause harm in a biological context.

Risk: The probability of that hazards of the material will have clinical adverse effects.

Appropriate in vitro tests are valuable.

Approval and re-approval in any level of tests would play a crucial role.

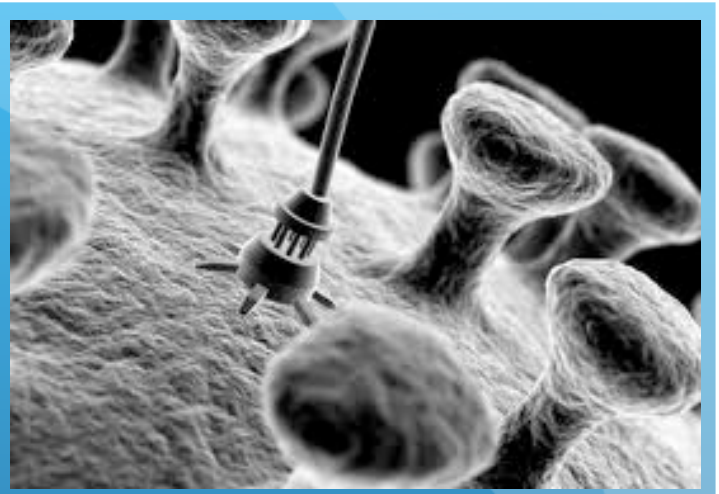
The evidence-base for exemption of not new or modified materials.



Hazards:
Chronic toxicity from ingestion
carcinogenicity
genotoxicity
and other safety-oriented test

risk:
Test for sensitization
Pulpal inflammation
Bone formation
and other cellular response

Conclusion



- Biomaterial
- Structural <-> Therapeutic
- The needs of biological testing will extend beyond safety.
- Efficient biological assessment for measure and predict Compatibility

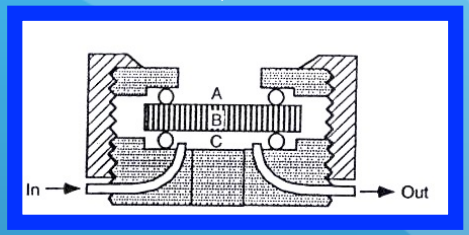
Correlation among In Vitro, Animal, and Usage Tests

- In the field of biocompatibility, some scientists question the usefulness of in vitro and animal tests in light of the apparent lack of correlation with usage tests and the clinical history of materials.
- However, the lack of correlation is not surprising in light of the differences among these tests.

Correlation among In Vitro, Animal, and Usage Tests

- In vitro and animal tests often measure aspects of the biological response that are more subtle or less prominent than in a material's clinical usage.
- Barriers between the material and tissues may exist in usage tests or clinical use that may not exist in *in vitro* or animal tests.
- It is important to remember that each type of test has been designed to measure different aspects of the biological response to materials, and correlation may not always be expected.

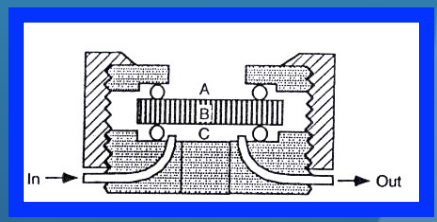
Correlation among In Vitro, Animal, and Usage Tests



- The best example of the barrier that occurs in use but not in vitro is the dentin barrier.
- When restorative materials are placed in teeth, dentin will generally be interposed between the material and the pulp.
- The dentin barrier, although possibly only a fraction of a millimeter thick, is effective in modulating the effects of dental materials.

Correlation among In Vitro, Animal, and Usage Tests

- The effect of the dentin barrier is illustrated by the following classic study.
- Three methods were used to evaluate the following materials: a ZOE cement, a composite material, and a silicate cement.
- The evaluation methods included
  - (1) four different cell culture tests
  - (2) an implantation test
  - (3) a usage test in Class V cavity preparations in monkey teeth



Correlation among In Vitro, Animal, and Usage Tests

- The results of the four cell culture tests were relatively consistent, with silicate having only a slight effect on cultured cells, composite a moderate effect, and ZOE a severe effect.
- These three materials were also embedded subcutaneously in connective tissue in polyethylene tubes (secondary test), and observations were made at 7, 30, and 90 days.
- Reactions at 7 days could not be determined because of inflammation caused by the operative procedure.
- At 30 days, ZOE appeared to cause a more severe reaction than silicate cement.
- The inflammatory reactions at 90 days caused by ZOE and silicate were slight, and the reactions to composite materials were moderate.

Correlation among In Vitro, Animal, and Usage Tests

- When the three materials were evaluated in class V cavity preparations under prescribed conditions of cavity size and depth (usage test), the results were quite different from those obtained by the screening methods.
- The silicate was found to have the most severe inflammatory reaction, the composite had the moderate to slight reaction, and the ZOE had little or no effect.

Correlation among In Vitro, Animal, and Usage Tests

- The apparent contradictions in this study may be explained by considering the components that were released from the materials and the environments into which they were released.
- The silicate cement released hydrogen ions that were probably buffered in the cell culture and implantation test but may not have been adequately buffered by the dentin in the usage tests.

Correlation among In Vitro, Animal, and Usage Tests

- Microleakage of bacteria or bacterial products may have added to the inflammatory reaction in the usage test.
- Thus this material appeared most toxic in the usage test.
- The composites released low-molecular-weight resins, and the ZOE released eugenol and zinc ions.

Correlation among In Vitro, Animal, and Usage Tests

- In the cell-culture tests, these compounds had direct access to cells and probably caused the moderate to severe cytotoxicity.
- In the implantation tests, the released components may have caused some cytotoxicity, but the severity may have been reduced because of the capacity of the surrounding tissue to disperse the toxins.

Correlation among In Vitro, Animal, and Usage Tests

- In usage tests, these materials probably were less toxic because the diffusion gradient of the dentin barrier reduced concentrations of the released molecules to low levels.
- The slight reaction observed with the composites may also have been caused in part by microleakage around these restorations.
- The ZOE did not show this reaction, however, because the eugenol and zinc probably killed bacteria in the cavity, and the ZOE may have somewhat reduced microleakage.

Correlation among In Vitro, Animal, and Usage Tests

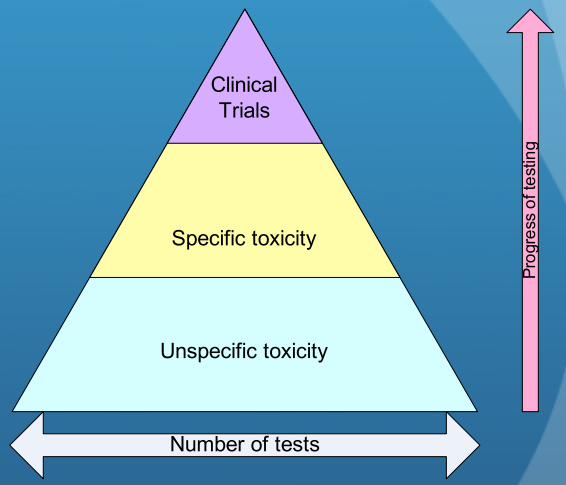
- Another example of the lack of correlation of usage tests with implantation tests is the inflammatory response of the gingiva at the gingival and interproximal margins of restorations that accumulate bacterial plaque and calculus.
- However, connective tissue implantation tests are of great value in demonstrating the cytotoxic effects of materials and evaluating materials that will be used in contact with alveolar bone and apical periodontal connective tissues.
- In these cases, the implant site and the usage sites are sufficient similar to compare the test results of the two sites.

Using In Vitro, Animal, and Usage Tests together

- Early combination schemes proposed a pyramid testing protocol, in which all materials were tested at the bottom of the pyramid and materials were “weeded out” as the testing continued toward the top of the pyramid (A).
- Tests at the bottom of the pyramid were “unspecific toxicity” tests of any type (in vitro or animal) with conditions that did not necessarily reflect those of the material’s use.
- The next tier shows specific toxicity tests that presumably dealt with conditions more relevant to the use of the material.
- The Final tier was a clinical trial of the material.

Using In Vitro, Animal, and Usage Tests together

- (A pyramid) The earliest strategy, in which the testing strategy is focused on toxicity only.
- Unspecific toxicity were tests not necessarily related to the use of the material, whereas the specific toxicity were more relevant.
- Clinical trials are equivalent to usage tests in this scheme.



Using In Vitro, Animal, and Usage Tests together

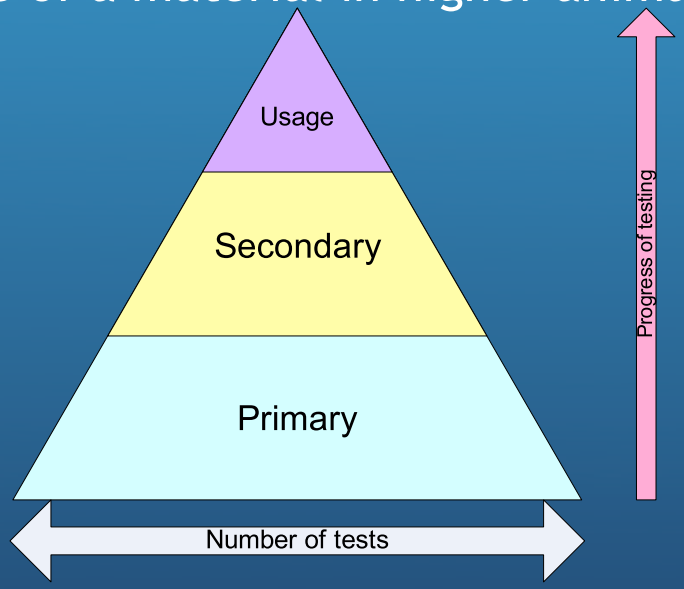
- Later, another pyramid scheme (B) was proposed that divided tests into initial, secondary, and usage tests.
- The philosophy was similar to the first scheme, except the types of tests were broadened to encompass biological reactions other than toxicity, such as immunogenicity and mutagenicity.
- The concept of a usage test in an animal was also added (vs. a clinical trial in a human).

Using In Vitro, Animal, and Usage Tests together

- There are several important features of these early schemes.
  - First, only materials that “passed” the first tier of tests were graduated to the second tier, and only those that passed the second tier were graduated to the clinical trials.
  - Second, any material that survived all three tiers of tests were deemed acceptable for clinical use.
  - Third, each tier of the system put a great deal of bonus on the tests use to accurately screen in or out a material.

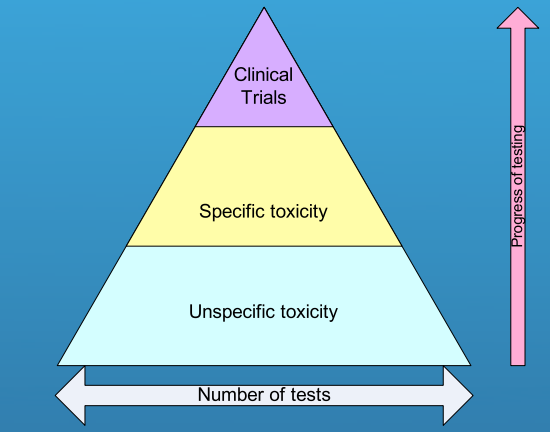
Using In Vitro, Animal, and Usage Tests together

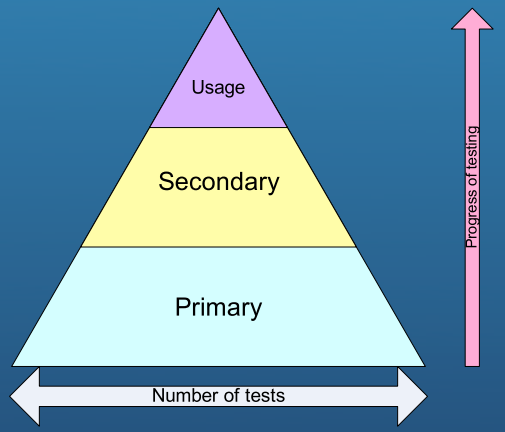
- Primary tests are in vitro and in vivo tests, but not necessarily related to the use of the material.
- Usage tests are either clinical trials in humans or a close model of the use of a material in higher animals.



Using In Vitro, Animal, and Usage Tests together

- In both of these testing strategies (A and B), the major problem is the inability of the early tests to accurately predict problems with the materials. Thus good materials might be screened out and poor materials might be advanced.





Using In Vitro, Animal, and Usage Tests together

- Two newer testing schemes (C & D) have evolved in the past 5 years with regard to using combinations of biocompatibility tests to evaluate materials.
- Both of these newer schemes accommodate several important ideas.

Using In Vitro, Animal, and Usage Tests together

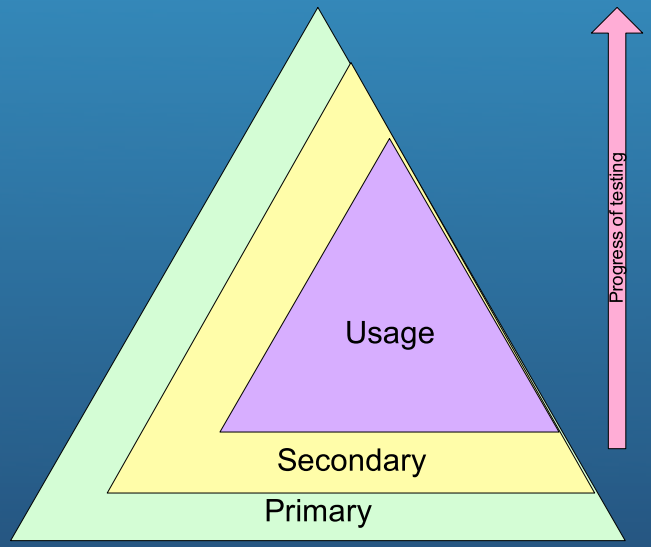
- First, all tests (in vitro, animal, and usage) continue to be of value in assessing the biocompatibility of a material during its development and even in its clinical service.
- For example, tests in animals for inflammation may be useful during the development of a material, but may also be useful after a problem is noted with the material after it has been on the market for a time.

Using In Vitro, Animal, and Usage Tests together

- Second, the newer schemes recognize the inability of current testing methods to accurately and absolutely screen in or out a material.
- Third, these newer schemes incorporate the philosophy that assessing the biocompatibility of a material is an ongoing process.

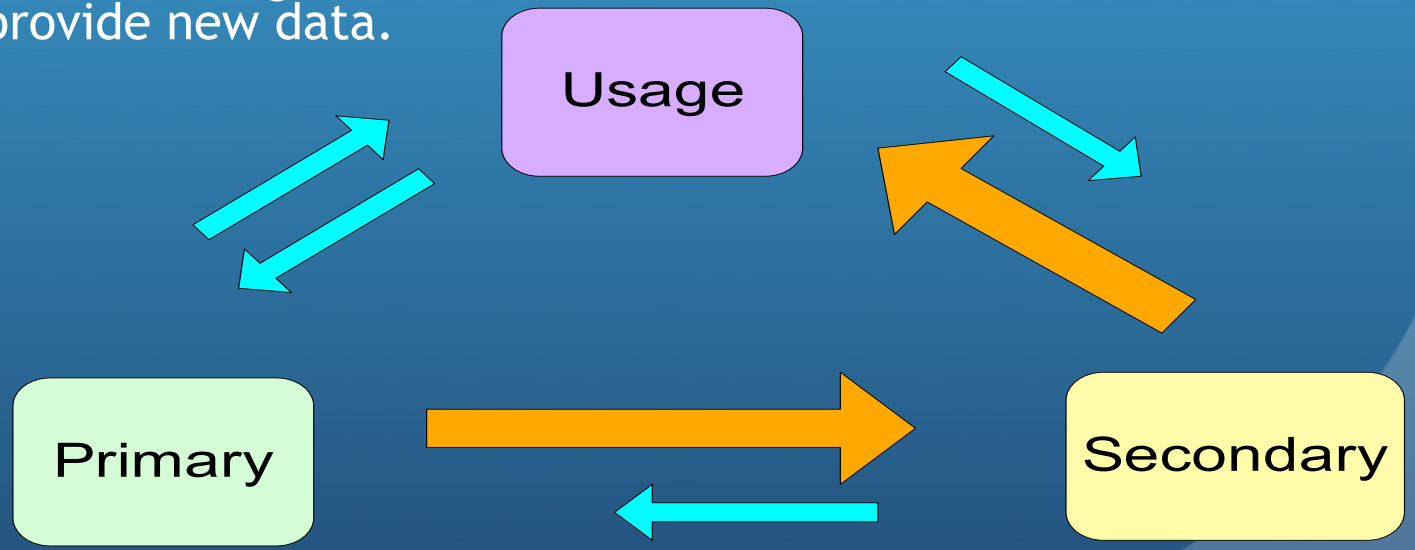
Using In Vitro, Animal, and Usage Tests together

- C. The pyramid scheme of A and B is retained, but it is acknowledged that primary and secondary tests will play a continuing (but decreased) role as the progress of the testing continues



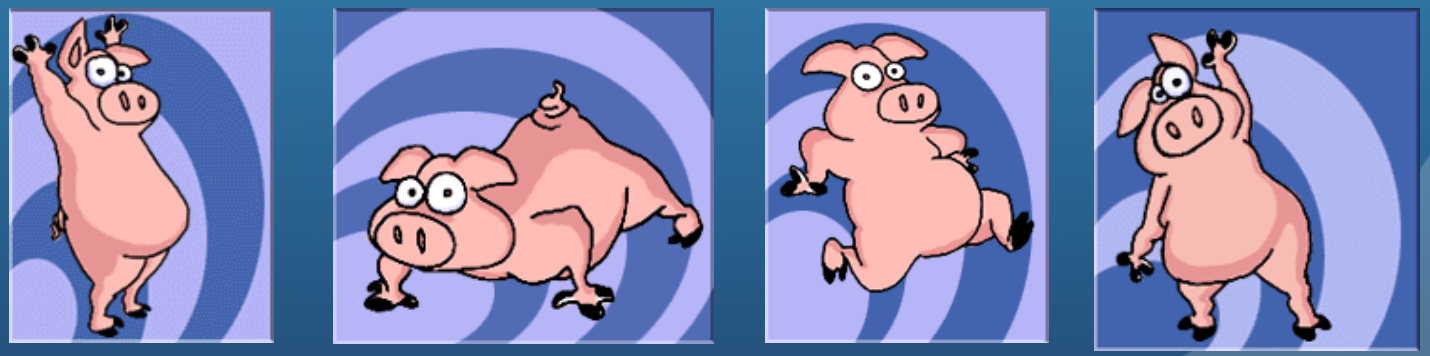
Using In Vitro, Animal, and Usage Tests together

- D. The ongoing nature of biocompatibility is recognized by the need to use primary and secondary tests after clinical evaluation of a material.
- In this scheme the order of testing is ultimately determined as the testing and clinical use of the material continue to provide new data.



Using In Vitro, Animal, and Usage Tests together

- Undoubtedly, we will see still newer strategies in the use of combinations of biocompatibility tests as the roles of materials change and the technologies for testing improve.



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