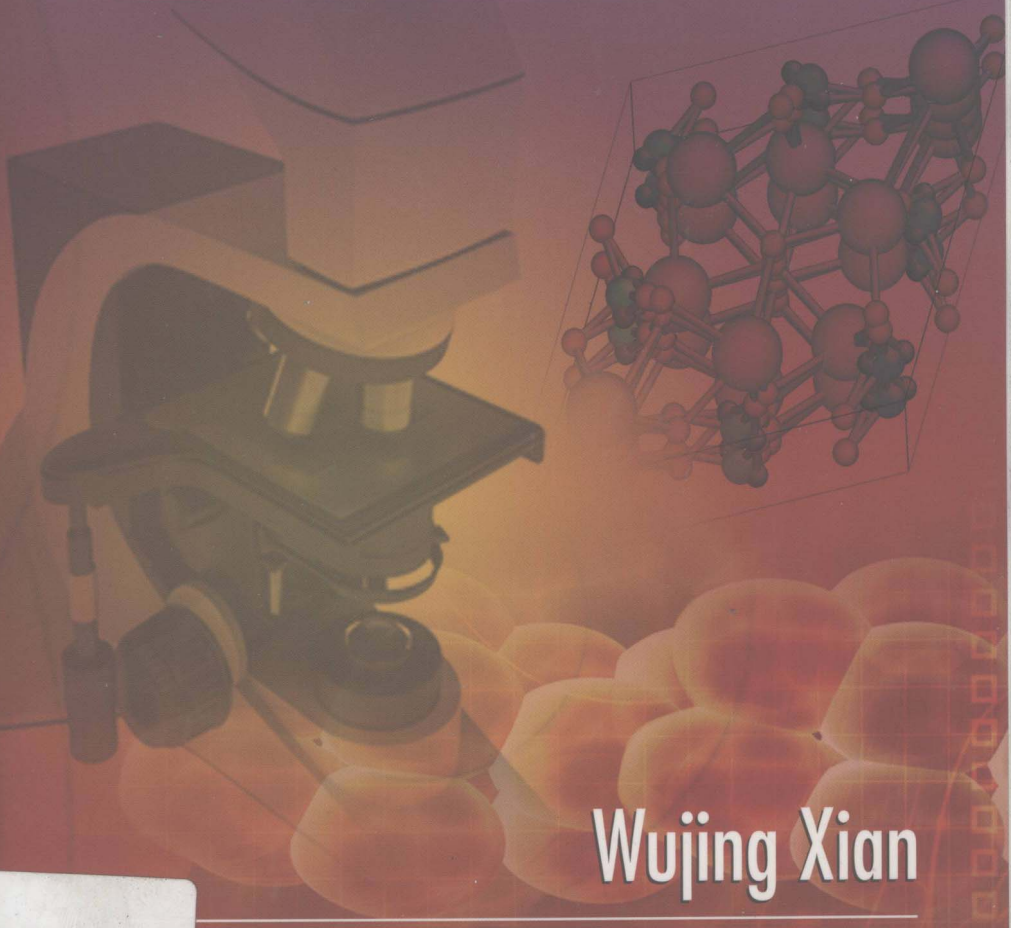
A Laboratory Course in BIOMATERIALS



Wujing Xian

CRC Press
Taylor & Francis Group

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CRC Press
Taylor & Francis Group
Boca Raton London New York

CRC Press is an imprint of the
Taylor & Francis Group, an **informa** business

CRC Press
Taylor & Francis Group
6000 Broken Sound Parkway NW, Suite 300
Boca Raton, FL 33487-2742

Printed in the United States of America on acid-free paper
10 9 8 7 6 5 4 3 2 1

International Standard Book Number: 978-1-4200-7582-3 (Paperback)

Library of Congress Cataloging-in-Publication Data

Xian, Wujing.
A laboratory course in biomaterials / Wujing Xian.
p. ; cm.
Includes bibliographical references and index.
ISBN 978-1-4200-7582-3 (pbk. : alk. paper)
1. Biomedical materials--Laboratory manuals. 2. Biomedical materials--Study and teaching. I. Title.
[DNLM: 1. Biomedical Engineering--Laboratory Manuals. QT 25 X64L 2009]

R857.M3X53 2009
610.28'4--dc22 2009011787

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Preface

The area of biomedical engineering has vastly expanded in the past couple of decades. The need to educate the multidisciplinary students who will make up the work force in research and industry in biomedical engineering has also correspondingly increased. A brief survey of bioengineering and biomaterials programs in a number of academic institutions reveals a general consensus on topics that are covered by lecture courses. Topics and other teaching aids for laboratory courses, however, are much less readily available. In fact, few biomaterials lab courses exist at present, despite the clear need. It is the intention of this book to provide a laboratory curriculum that is comprehensive in scope as well as current in its perspective. This book is suitable for an undergraduate laboratory course in biomaterials, and bioengineering at the senior or junior level. It is also designed to help lower the barriers for entry into biomaterials for the more "traditional" engineering departments (such as materials science, chemical engineering, and mechanical engineering) since the cost in time and resources required for developing such laboratory courses can be quite high. This course is inherently multidisciplinary, integrates a variety of principles from materials science, mechanical engineering, chemistry, biochemistry, molecular and cell biology, and tissue engineering, and will train students in laboratory skills, data analysis, problem solving, and scientific writing. Experiments in this course are described in the form of flexible modules that can be chosen and adapted for the needs of different departments. Within each module, a range of multidisciplinary knowledge and laboratory practices are organized around a central theme, so that students can see the labs not as a compilation of procedures but rather as a coherent whole consisting of interconnections from various disciplines. Much has happened recently in this dynamic field; many experiments in this lab course are adapted from research papers and reflect recent progress in bioengineering and biomaterials.

As a laboratory manual, this book provides step-by-step descriptions of lab procedures, reagents, equipment, and even data processing guidelines so that a laboratory course can be started from scratch. These descriptions are guidelines rather than rigid prescriptions, and the experiments can be adapted according to the instructional laboratory settings and the students' learning needs. The questions following each module incorporate some of the frequently encountered problems and mistakes made by the students from my own teaching experience. Finally, it is my hope that this laboratory course is a fun and rewarding experience for students as well as teachers. Let's get started!

Acknowledgments

This book is based on the biomaterials laboratory course that I have taught for several years at the Materials Science and Engineering Department (MatSE) at the University of Illinois at Urbana-Champaign. I am very grateful to the people who have helped me with the course and with writing the book. Thanks are in order to Professor J. J. Cheng for the many helpful discussions; to Dr. Joanne Manaster, Sheeny Lan, Dr. Aylin Sendemir-Urkmez, and Dr. Sharon Wong for their help with cell cultures; to Dr. Raju Perecherla and Professor David Cahill for access to and help with instruments; to Spencer Shultz for his expert machine-shop work; and to Zach Culumber, Lanfang Li, Scott Slimmer, and Lihua Yang for their assistance. I am also thankful for the commitment to and support for biomaterials by my department. Especially I'd like to thank Jay Menacher, assistant to the head of MatSE, who has made things easy. And lastly, I am deeply grateful to Gerard Wong, who suggested that I write this book and helped with his expertise and moral support along the way: This book would not have been possible without him.

About the Author

Wujing Xian received a BS degree in chemistry from Sun Yat-Sen (Zhongshan) University, and a PhD degree in chemistry from the University of Nebraska–Lincoln. After postdoctoral work on protein structure and engineering at the Brigham & Women's Hospital of Harvard Medical School and the Institute for Medicine and Engineering at the University of Pennsylvania, Dr. Xian became a lecturer at the University of Illinois at Urbana-Champaign, where she created a new biomaterials laboratory course for the Department of Materials Science and Engineering. Her research interests include protein engineering, tissue engineering, and antimicrobials.

Contents

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Basic Laboratory Skills I

Experiments in this course aim to provide lab skill training in many disciplines. Before we begin, however, it is helpful to familiarize ourselves with some of the basic lab practices so that we can conduct the experiments more efficiently and safely. To get started, we will survey some of the most commonly used equipment and supplies found in our lab. We will also learn about proper waste disposal, an often ignored but extremely important practice that ensures protection of both the environment and ourselves. Next we will learn basic practices in liquid transfer and weighing; then, to integrate these practices, we will make two solutions that will be later used in our experiments. Last, we will review some of the basic practices in data processing.

I Commonly Used Lab Equipment and Supplies

- **Balances.** Top-loading electronic balances are commonly used nowadays. An electronic balance can be categorized as either *regular* or *analytical* depending on its weighing resolution, or "readability." A regular balance typically has a range of hundreds to thousands of grams and readability from milligrams to grams, while an analytical balance's range is typically of tens to hundreds of grams and its readability is ≤0.1 mg. When weighing samples, choose the right balance based on the weight of the sample and the accuracy requirement. A special type of mechanical balance, the trip balance, is also used in lab. (See Section IV for more details.)
- **Centrifuges.** There are a variety of centrifuges for different centrifugation needs, and one of the determining factors for centrifuge selection is the centrifugal force that is required for sedimentation of components in the sample. Typically, mini-centrifuges and microcentrifuges are used for quick processing of small volumes of samples (typically <2 ml each), whereas larger centrifuges, with either bench-top or floor models, accommodate larger volumes and offer more centrifugal power.
- **Glassware.** Glass beakers, Erlenmeyer flasks, test tubes, and bottles of different volumes are examples of typical glassware used in lab. To clean glassware, first wash with detergent until clean—water will flow as a sheet rather than streaks on clean glass surface—then rinse with water, and finally rinse with de-ionized water.
- **Gloves.** Disposable latex and nitrile gloves are the most commonly used in labs. Latex gloves are relatively inexpensive but have poor chemical resistance. Nitrile gloves have much better chemical resistance and should be worn when handling organic solvents or caustic reagents.
- **Liquid transfer.** Liquid transfer is handled in many different ways depending on the liquid volume and the accuracy requirement. Micropipettes, electronic

pipette-aids, transfer pipettes, and graduated cylinders are some of the most commonly used liquid transfer tools. (See Section III for more details.)

- **pH meter.** This is used for measuring the pH of solutions. Operate your pH meter according to the user's manual. It should be calibrated using pH standard buffers from time to time. *Important:* Do not allow the electrode to dry.
- **Mixing.** Vortex mixers, magnetic stirring plates, and shakers are examples of commonly used mixing equipment in the lab. In our experiments, we will also use specialized mixing equipment such as the sonicator and the homogenizer.
  - **Vortex mixer.** The high-speed vibration of a vortex mixer generates vortex in liquid, which results in quick mixing of liquid-liquid or liquid-solid. It is generally used for quick mixing in microcentrifuge tubes, test tubes, etc., in which vortexes can be generated.
  - **Magnetic stirring plate with stirring bar.** This is generally used for mixing in beakers, flasks, bottles, or other similar containers. For efficient stirring, the length of a magnetic stirring bar should be about 2/3 the diameter of the container's bottom.
  - **Shaker.** This is suitable for consistent mixing for prolonged periods of time. Some shakers are equipped with environment control such as water bath that can maintain constant temperature for incubating and shaking.
- **Refrigeration.** Some reagents need to be stored at low temperatures. For a given reagent, refer to the on-bottle label, the user's manual, or the material safety data sheet (MSDS) for the appropriate storage temperature.
- **Water purification.** Requirement of water purity varies depending on the application. Water purity is categorized as type I, II, and III, which typically requires the resistivity to be >18.2 mΩ·cm, >1 mΩ·cm, and >50 kΩ·cm, respectively; other parameters are restricted as well. For reagent preparation, type I water is generally preferred. On the other hand, for less demanding usage of purified water such as glassware rinsing, type III water is usually sufficient. (Note that de-ionized water can be corrosive to metal, thus it may not be suitable for certain applications.) De-ionization can be achieved by filtering water through mixed-bed ion-exchange resins that remove both anions and cations.
- **Disposable items.** Commonly used disposable items include 0.5-ml and 1.5-ml microcentrifuge tubes, pipette tips, serological pipettes (polystyrene), weighing boats, flint glass test tubes, etc. Observe waste disposal guidelines when discarding these items.

Exercises

1. Survey the centrifuges in your lab using Table 1.1 as an example.
2. Locate a vortex mixer, a magnetic stirring plate, and a shaker in your lab.
3. What type(s) of water purification equipment is(are) available in your lab? In your lab notebook, record the make and model of the equipment, the type of water (I, II, or III), and the resistivity threshold of the water purifier.

TABLE 1.1

A Survey of the Centrifuges in My Lab

Make	Model no.	Type[a]	Rotor	Max. RPM[b]	Max. RCF[c]	Vol. Capacity
Fisher Sci.[d]	—	Mini	Fixed-angle	6600	2200 ×g	6 × 2.0 ml
			Strip	6600	2200 ×g	8 × 0.2 ml or 16 × 0.2 ml
...	...	...	...	...	...	...

[a] Centrifuge type can be minicentrifuge, microcentrifuge, general-purpose centrifuge (bench-top or floor model), clinical centrifuge, etc.

[b] RPM: rotation per minute.

[c] RCF: Relative centrifugal force, expressed as a number of times of gravity, or "×g."

[d] Example (Fisher cat. no. 05-090-100). This model includes two rotors.

II Waste Disposal

It is important to observe applicable institutional guidelines when disposing of used supplies and reagents. Always ask your instructor if you are not sure how to dispose of a certain item. The following are some of commonly available waste disposals:

- **Broken glass disposal.** Clean, nonhazardous broken glassware, Pasteur pipettes, disposable glass tubes, etc. should be collected in a designated plastic-lined box clearly marked as "broken glass disposal." Residual chemicals or reagents should be removed from the glassware before disposal. *Example:* A Pasteur pipette was used for transferring chloroform. Before discarding it into broken glass disposal, leave the pipette in a fume hood until the residual chloroform has completely evaporated.
- **Regular trash.** Some disposable items such as plastic serological pipettes, plastic transfer pipettes, paper tissues, etc. that have not been used with toxic, caustic, or carcinogenic reagents may be discarded into regular trash. *Example:* A plastic transfer pipette was used for adjusting pH with HCl solution. Before disposing it into regular trash, rinse the pipette with water to remove any residual HCl solution.
- **Sharps disposal.** Sharps such as scalpels, blades, syringes, and needles need to be disposed of in special heavy-duty plastic sharps containers, which should be clearly marked. (Syringes, though made of plastic, are often required to be disposed of in sharps containers because of the association between syringes and needles.) *Example:* A scalpel was used for cutting up calf skin. When finished, wipe it on a piece of tissue paper to remove any stuck skin tissues and discard it into a sharps container.
- **Sink disposal.** Institutional and local municipal guidelines must be strictly followed for sink disposal. In general, only small amounts of nonhazardous inorganic salts, acids, and bases can be flushed down the sink with a large amount of water. *Example:* In our experiments, phosphate-buffered saline, electrophoresis running buffer, acetic acid solution, dilute HCl solution, NaOH solution, and excess salts such as NaCl taken from the bottles when weighing samples, etc. can generally be flushed down a sink with a generous amount of water.

- **Disposal of solvents, reagents, and chemicals.** These should be collected in specially designated containers and disposed of following proper guidelines. *Example:* 70% ethanol is used for disinfecting calf skin before collagen extraction. When finished, the 70% ethanol should be collected in a container clearly labeled with "70% ethanol waste with trace calf skin" or similar wording.

Exercise

Locate the broken glass disposal and sharps disposal containers in your lab.

III Liquid Transfer

III.1 Micropipettes

Micropipettes, also called micropipetters or pipettes, are piston-driven air-displacement devices that are typically used to transfer liquid volumes in the range of 1 µl to 1000 µl with high precision and accuracy, which can be as low as <0.5% or as high as 5% depending on the micropipette and the pipetted volume. Specialized micropipettes are available for larger or smaller volumes.

III.1.1 Anatomy of a Micropipette

Different micropipettes may have different designs, but they all have certain common features and are operated in similar ways:

- The handle. Grab the handle firmly with your hand.
- A push button, or plunger, on the top of the micropipette. Use the thumb to push the plunger for delivery of the liquid. There should be two stops when pushing down on the plunger. Usually, for delivery of the bulk volume, press the plunger to the first stop and to deliver the residual liquid, press the plunger to the second stop.
- A volume adjustment dial and a numeric display. Turn the dial to set the volume of the liquid to be delivered. For accuracy, turn the dial past the desired setting, and then turn it back down gradually to the correct setting. The unit for the set volumes is µl; make sure that the decimal place is located correctly on the dial.
- A shaft that leads to the tip cone. To attach a tip to the tip cone, firmly push down the pipette (but avoid using excessive force).
- A tip ejector. To eject a tip, point the tip to the waste receptacle and press the ejector with your thumb.

III.1.2 Pipetting Techniques

Observe the following guidelines when using a micropipette:

- Pick the right micropipette. Do not use a micropipette outside its designated range; otherwise it could be damaged mechanically. When a volume to be pipetted is within the ranges of different micropipettes, use the one with the smallest range.

For example, to pipette a volume of 18.2 µl, use a 20-µl micropipette instead of a 100-µl micropipette.

- *Never* allow liquid to get inside the micropipette barrel. This will lead to cross-contamination between samples, and potential damage to the micropipette as well. To avoid this situation, pipette liquid in smoothly, and do not tilt the micropipette too far from vertical when it is holding liquid.
- Press and release the plunger smoothly. Do not allow it to snap back.
- When loading the pipette tip onto the tip cone, make sure that the tip is firmly attached but avoid using excessive force—a light tap or two on the tip rack will usually do.
- Pipetting in: Submerge the pipette tip slightly (several mm) beneath the surface of the liquid. Do not plunge the tip into the liquid.
- Pipetting out: Hold the tip against the inner wall of the receptacle for a steady and smooth delivery of the liquid.

Select the following pipetting techniques based on the volume of the liquid to be transferred, and whether it is nonviscous, viscous, or foamy:

- **The forward technique.** The forward technique is typically used for nonviscous liquid. To pipette using the forward technique, press the plunger to the first stop and release to draw liquid in through the tip. Wipe the tip against the liquid container to remove excess liquid on the outside of the tip. In the new receptacle, press the plunger to the first stop to deliver the liquid. Wait for a second or two, and then press the plunger to the second stop to empty any residual liquid. Release the plunger and eject the tip.
- **The reverse technique.** The reverse technique is suitable for viscous or foamy liquid, or very small volume. Examples of viscous or foamy liquids include glycerol solutions, protein solutions, and detergent solutions. To pipette using the reverse technique, first press the plunger all the way to the second stop, then release slowly to draw liquid in. Again, wipe the tip against the liquid container to remove excess liquid on the outside of the tip. To deliver, press the plunger to the first stop and hold for one or two seconds. The remaining liquid should be released back into the original liquid container or discarded.
- **The pumping technique.** Some liquids, such as whole blood, are viscous and tend to cling to pipette tips. Repeated pumping is necessary to deliver the full volume. To pipette using the pumping technique, first press the plunger to the first stop, then release to draw up liquid. Press the plunger to the first stop to deliver the liquid, and then release it smoothly. Repeat the pumping motion until all the liquid inside the tip is delivered. To finish, press the plunger to the second stop to deliver any residual liquid.

III.1.3 Before You Put Away the Micropipette ...

After work is finished, do the following before you put the micropipette away for the day:

- Clean the micropipette. Wipe away any moisture or soiled spots. If the micropipette has been used to handle hazardous materials, make sure that no residue is left behind.

- Turn the volume adjustment to the highest setting. This is to prevent the spring inside the micropipette from being compressed for a long period of time, which helps to maintain the accuracy of micropipette.
- For small-volume micropipettes (<10 µl), it is recommended that they be stored with pipette tips attached, so that in case the micropipettes are dropped, their small tip cones are protected.

III.2 Pipet-Aids

A pipet-aid is a device that is used with a serological pipette to transfer liquid volumes from <1 ml to ~100 ml. Commonly used pipet-aids are electrical and are controlled by two buttons: Press the top button to draw up liquid and the down button to deliver the liquid. A serological pipette is attached to the mouthpiece of a pipet-aid.

Pay attention to the following when using a pipet-aid for our experiments:

- ***Caution:*** Do *not* draw liquid into the mouthpiece of the pipet-aid.
- Pipetting organic solvent: To pipette organic solvents, glass serological pipettes *must* be used. Polystyrene pipettes will dissolve in many organic solvents. To tell a glass pipette from a polystyrene pipette: 1) read the label on the pipette; 2) feel the pipette: glass pipettes are heavier and clink when bounced on a hard surface. Pay attention to whether the glass pipette is reusable or disposable.
- Pipetting in: Liquid should be drawn up smoothly; do not allow it to "spring" (like spring water gushing out of the ground) through the opening.
- Pipetting out: Rest the tip of the serological pipette against the inner wall of the receptacle if possible, and deliver the liquid smoothly.

III.3 Pasteur Pipettes

Pasteur pipettes are glass pipettes with long, fine tips that need to be attached to rubber bulbs for pipetting. These pipettes are inexpensive and have excellent chemical resistance; they are especially suitable for transferring organic solvents and reagents when volume accuracy is not required. ***Caution:*** Pasteur pipettes should be disposed of in broken glass disposal.

III.4 Plastic Transfer Pipettes

These are disposable plastic pipettes with "built-in" suction bulbs. They can be used to transfer small volumes of liquid (<10 ml each transfer) when volume accuracy is not required. (They are also very useful for other tasks; for example, a transfer pipette can be used for resuspending a pellet after centrifugation since it can be used as a stirring rod as well as a pipette.)

III.5 Graduated Cylinders and Volumetric Flasks

Graduated cylinders and volumetric flasks are liquid-measuring devices that are mainly used for making solutions. Volumetric flasks are used when the accuracy requirement for