Methods for the characterization and evaluation of drug-device combination products

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11.1 Introduction

The purpose of this chapter is to provide the reader with a brief description of drug-device combination products and how they are regulated. As these products contain both a drug and device component, their performance testing necessarily combines elements of the essential requirements for both. Moreover, the combined product itself will possess novel attributes that will require testing to ensure the impact of the one component upon the other is well understood. Understanding such nuances will help product developers streamline their processes and facilitate a faster and smoother route to market by avoiding common mistakes, reducing or eliminating rework and being prepared for the types of questions that will be raised during discussions by regulatory bodies.

11.2 What is a combination product?

In order to significantly advance patient care, innovative technologies are required that will bring about a step-change in the performance of the medical products of the future. Combination products offer one approach to convey such a promise. They comprise two or more regulated components (e.g., drug-device, biologic-device or drug-biologic) either physically, chemically or otherwise united in one product, in an effort to provide synergistic benefits from their combined properties. These products come in a wide variety of different formats, for instance combined together in the packaging as pre-filled syringes containing drug or biologic, metered dose inhalers, transdermal patches, drug-laden wound dressings, or implantable combinations such as surgical sealants or drug eluting stents (DES). In some cases (as in the US) they may still be classed as combination products even if the two components are co-packaged, such as for needless injectors or delivery pumps plus a drug/biologic sold in the same pack or separately packaged and cross-labeled. They can be therefore be broadly considered under three types:

- Single-entity (e.g., integral):
  Prefilled drug delivery systems such as prefilled syringe, autoinjector, inhaler, patch
  Medicated devices such as drug eluting stents and balloons, antimicrobial mesh

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- Co-packaged (e.g., kits):
  Convenience kits such as surgical tray with anesthetic, vial and syringe packs
  Spinal fixation cage, collagen sponge with bone morphogenetic protein
- Co-labeled (e.g., virtual):
  Companion diagnostics, contrast agent and diagnostic imaging, photodynamic therapy.

Combination products are not new and have in fact been around since the medical devices amendments act of 1976; but it is only since the turn of the millennium that some regulatory bodies have provided guidance to industry on the development of these products. This has been driven primarily from the increased activities of both the medical device and pharmaceutical industries in creating products primarily for implantation that are capable of drug delivery for the local treatment of specific biological sequelae or disease states. The therapeutic agent may for instance, ameliorate an adverse biological response that ensues as a consequence of the implantation or presence of the device; for example an anti-inflammatory agent delivered from endocardial pacemaker electrodes is added to reduce fibrous connective tissue formation, resulting in the need for lower voltage stimulation thresholds and also aiding in its eventual removal from the body (Levy et al., 1990). This approach therefore overcomes deficiencies in the device performance related to biocompatibility or the body's reaction to the implant, which cannot be overcome by changes in the engineering design alone. Similarly, the active agent may be selected to guard against any subsequent infection following placement of the device (as for antibiotic-containing orthopedic cements (Passuti and Gouin, 2003)). Alternatively, the active agent can be present to address a pre-existing disease, as is the case for embolic drug eluting beads; arteries feeding a tumor are primarily physically occluded by intra-arterial administration of the device, which can then subsequently deliver a sustained and local dose of chemotherapy to the diseased and dying tissue (Lewis, 2009). Thus, combination products can offer significant clinical benefits in terms of enhanced device performance, with improvements in drug efficacy and safety by virtue of high local concentrations and lower systemic exposure.

This chapter will discuss some of the considerations that should be made when planning the pre-clinical testing of combination products, with a specific focus on drug-device combinations.

11.3 How are combination products regulated?

Clearly there is an abundance of new considerations for the combination product for which the application of conventional product safety and efficacy testing, as outlined throughout this book, may be insufficient. The specific requirements may depend upon the office to which the combination product is assigned for review; each component will have a different formal regulatory pathway in itself, which will impact greatly on all aspects of the product development, approval and commercialisation. There will of course be some common ground and typically information on laboratory testing, animal studies, stability testing, clinical study design, long-term follow-up and post-approval data may need to be provided. The perspective of the different agencies

reviewing the data however, may be quite different and hence care must be taken in consultation with all involved, on what constitutes an acceptable approach to satisfy all of the requirements. It is ultimately the developer's obligation to perform a risk assessment in order to guide the decisions as to the appropriate testing. A detailed account of the respective regulatory requirements for combination products is therefore outside the scope of this chapter; the reader is referred to Chapters 18 (S. Leppard) and 19 (J. Greenbaum) of the book: drug-device Combination Products-Delivery Technologies and Applications, which review European and US regulatory aspects in great detail (Lewis, 2010).

11.4 Demonstrating safety and efficacy of combination products

There are numerous guidance documents available to help determine the requirements for demonstrating safety and efficacy of combination products (Administration FaD, 2006; Portnoy and Koepke, 2005a,b; Donawa, 2008a,b), but depending upon the degree of innovation of any new technology, these principles may need to be significantly adapted. Hence, the developers of such products are encouraged to consider not only the component parts but particularly the issues raised by placing them in combination. This should ensure the generation of a comprehensive and inclusive approach without the need to perform studies that become superfluous by virtue of the combination.

11.4.1 Combinations composed of one or more previously approved/cleared components

The most ideal place to start with the development of a combination product is one in which one or both of the constituent parts have been previously approved and/or cleared for use in the indication for which the combination product is proposed. This avoids a great deal of testing and will streamline the development process. The key however, is to recognize what new issues are now raised as a consequence of combining the components. Fig. 11.1 illustrates some of these considerations as set out in the Guidance for Industry and FDA Staff on Early Considerations for Innovation Combination Products (Administration FaD, 2006).

Clearly, the issues may not be simply about how the components may interact when combined, but also about matters such as potential changes in the proposed use of the combination product, the route by which it is administered, changes in local or systemic exposure to drug component, or type of patient population to be treated. Indeed, a product used to treat two different indications may be physically the same in each case but classified differently depending upon its primary mode of action in each indication.

In the case where the drug component is a new molecular entity (NME), it is necessary to firstly execute the conventional pre-clinical evaluations required to establish its safety, followed by first in man studies of the NME itself, before embarking upon

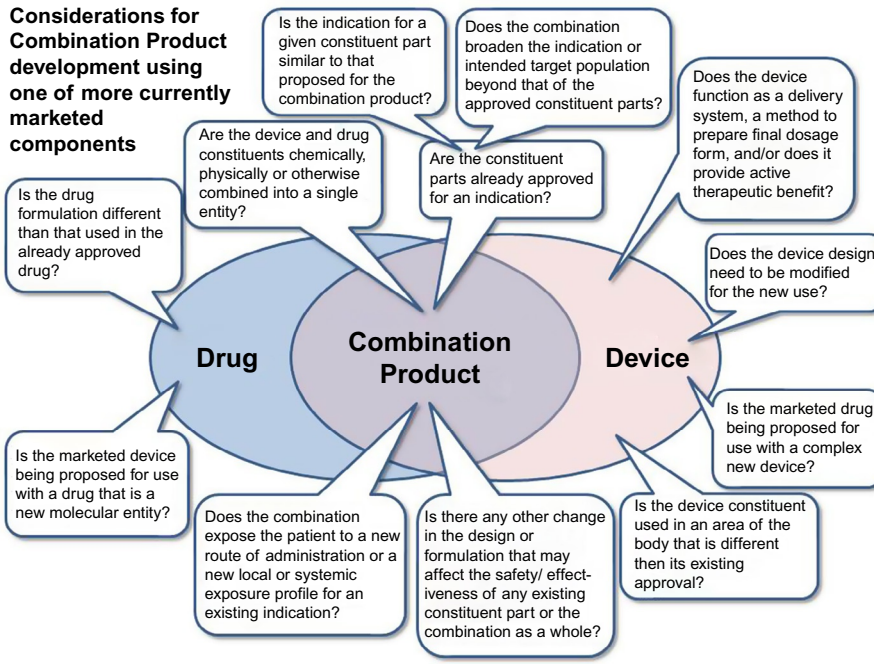


Fig. 11.1 Considerations for combination product development using one or more currently marketed components.

additional studies to assess the combination product. Careful planning is essential, as some of the tests that might be required, such as reproductive toxicity or carcinogenicity studies, are long-term in nature but they may be undertaken in parallel with some of the early clinical investigations if the risks are appropriately mitigated.

11.4.2 Evaluation of the interactions between the combination product component parts

When combining device and drug components, there is the potential for interaction. Indeed, certain interactions may be desirable; for instance, the device may be used to control the release of an active agent in order to sustain its delivery over a prolonged period (Lewis, 2010), or the combination may promote stability of the drug in its most active form (Tang et al., 2008). It is therefore important to understand the following:

- Whether the drug stability is changed when combined with or delivered from the device;
- Are there physical or chemical drug-device interactions that alter the dose of the drug delivered, for example by a catalyzed drug degradation or adsorption of the drug to the device;
- Does the presence of the drug interfere with the mechanical function of the device, or promote degradation of any part of the device;
- Are there leachables or residuals from manufacturing of the device that can interact with the drug or pose a safety issue upon their release;
- Does the device have an action at the point of use that could change the characteristics of the drug (for instance, as for photodynamic therapy where this is desirable).

Hence, robust validated methods for extraction and analysis of both the drug and device components may be required in order to detect such potential changes. Chromatographic (or other separation) methods that are able to separate pure drug from impurities and degradants are traditionally used (Aubry et al., 2009). High pressure liquid chromatography (HPLC), for instance, is a mainstay technique that is utilized to measure both dose and purity of components with accuracy and precision. Furthermore, physical methods will be needed to measure the influence of the drug on the physicomechanical properties of the device.

11.5 Pre-clinical testing of combination products

Broadly, the pre-clinical (or sometimes referred to as non-clinical) testing of a medical device essentially evaluates safety using a number of endpoints which include the overall biocompatibility of the device (for instance, as per recognized standards such as ISO10993), component testing (e.g., by ASTM methods), the appropriate selection and qualification of the raw materials used in the construction of the device and the final design validation testing prior to regulatory submission. For a drug-device combination product the pre-clinical testing package may need to be broadened in order to address new safety concerns introduced by the incorporation of the drug and the evaluation of its pharmacodynamics.

11.5.1 In vitro methods for bench testing

In vitro bench testing provides the first assessment of the combination product performance (Willis and Lewis, 2008a,b). Where the product exists in multiple configurations, for example the different lengths and diameters of DES, or the various calibrated size ranges of embolic drug eluting beads (DEB), the amount of product testing required can quickly expand exponentially. Bracketing is a common approach to reducing the testing burden, where the extremes of a particular parameter are chosen and a justification made that everything in between is the same. This relies on the parameter either remaining constant, or varying predictably across a range; but it may also be a risky approach if one of the extremes fails testing throwing the outcome of such a matrix in doubt. Clearly, given the myriad of sizes, shapes and uses of combination products, new bench tests may need to be developed in order to provide relevant information about the performance of the product in its proposed mode of application.

11.5.1.1 Physicomechanical testing

As drug-device combination products contain a device element which normally has a physical function, physicomechanical testing is usually required. This ensures that the device maintains its physical integrity for the duration of its expected use. In combining the drug and device elements, some adverse interaction may occur which could lead to early physical failure of the device component (as per Section 11.4.2), for instance, the inclusion of an antibiotic into an orthopedic cement can influence how the material sets and impact on its subsequent physical ability to withstand mechanical load (Marks et al.,

1976; Provenzano et al., 2004). Physicomechanical performance tests are devised to measure these effects and allow for engineering solutions to be applied to design around any issues. Conventional mechanical testing equipment, such as dynamic mechanical analysers, tensometers and indenters can be applied to measure a wide range of mechanical properties including tension, compression, flexure, fatigue, impact, torsion and hardness.

Case study: Embolic drug eluting beads

The resistance to compression for embolic DEBs is an important parameter which dictates whether the product can be delivered through microcatheters, which can often possess inner lumen diameters smaller than the beads and hence require the product to deform during delivery. Moreover, once administered into an artery, the beads will travel through the vessel until they physically occlude by virtue of their size; here, if the beads deform too much they can be squeezed too distally down a vessel or potentially pass through the capillaries and into the venous circulation where they could induce non-target embolisation. Whilst the deliverability of the product is best tested using a relevant microcatheter delivery use test, physicomechanical investigations have been applied in during their development using an Instron tensiometer to perform a modified test which measures the force to compress a sample of a number of beads held on a platter. This test was sensitive enough to demonstrate that drug loading significantly increased the force required to compress the beads, but was incapable of producing reproducible data for the smaller bead sizes (Lewis et al., 2007; Taylor et al., 2007). Since those early reports, new technologies have been developed, such as that offered by Femtotools GmbH which uses highly sensitive force sensing probes capable of measuring single bead compression across all sizes of product (Fig. 11.2).

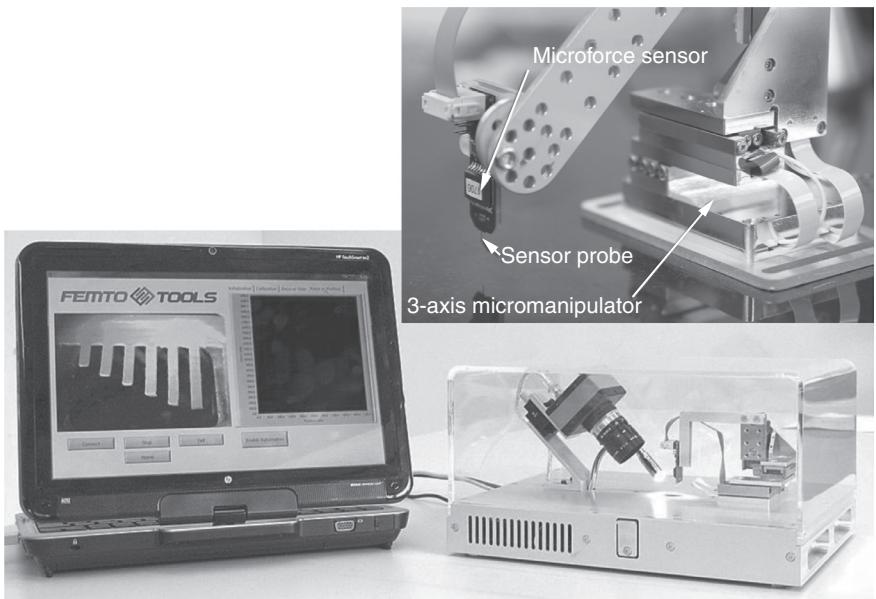


Fig. 11.2 Example of a microforce sensing apparatus for physicomechanical analysis of micro-scale devices or thin coatings.
Photographs courtesy of Femtotools GmbH, Zurich, Switzerland, www.femtotools.com.

Moreover, testing methods have evolved to be more relevant to the product indication and measures of rate of elastic recovery are also now possible which better mimic the performance of the product in vivo (Caine et al., 2017, 2018; Hidaka et al., 2010, 2011).

Companies such as Instron and Bose have opted to develop and commercialize a wide range of device-specific testing equipment which can be used to evaluate such properties as plunger forces for needles and syringes, fatigue and durability of DES or stent grafts, strength and stiffness of metallic wires and tubing, compression and strength of breast implants or wear and stress in orthopedic implants. These tests are aimed at demonstrating the device component maintains its primary function, and can often be performed according to recognized American Society for Testing and Materials (ASTM) guidance, some examples of which are listed in Table 11.1 for a selection of medical devices.

11.5.1.2 Evaluation of drug delivery coatings

One strategy for combining a drug with device is by use of a polymeric coating into which the active agent can be dispersed, and from which release can be subsequently modulated. Examples of combination products that utilize this approach include coronary stents that elute anti-restenosis drugs, anti-inflammatory-eluting hernia meshes or endotracheal tubes that release antimicrobial agents. Regardless of the application, the coating must be mechanically robust and durable to withstand cracking or delamination from the device during handling and implantation; it must also be stable to withstand the physical rigors of the device function and to act as a reservoir for the desired period of drug release (Willis and Lewis, 2008a). Once the drug has gone, the coating may then optionally remain as a bioinert component of the device, or biodegrade; the testing required to demonstrate safety of these two formats may therefore be quite different. The coating may consist of multiple layers that serve different purposes, such as topcoats that act as barrier layers to drug diffusion (as for the poly(butyl methacrylate)

Table 11.1 Selection of American Standard Test Methods for medical devices.

ASTM	Test
ASTM F382	Flexural fatigue testing metallic bone plates and fixture devices
ASTM F384	Metallic angles orthopedic fracture fixation devices
ASTM F543	Torsion testing metallic bone screws
ASTM F1714	Wear of prosthetic hip designs
ASTM F1717	Spinal constructs: static testing, fatigue testing
ASTM F2079 and F2477	Stents, tensile strength testing
ASTM F2256	Strength properties of tissue adhesives by T-Peel testing
ASTM F2346	Characterization and fatigue of intervertebral disc prostheses
ASTM F2606	Three-point bending balloon expandable vascular stents and stent systems

top-coat on the Cipher® DES), or as subbing layers that help to bind the coating to the substrate (for example, Parylene C is often selected for this reason). Whatever the format, the primary purpose of the coating is to act as a carrier for the active substance and release it in a controlled fashion. The evaluation of such coating systems therefore requires an understanding of each of the drug, coating and device component parts and crucially, how they interact with one-another (Forster et al., 2008a,b).

Drug analyses

The choice of drug or drug type used in a combination product is driven by the underlying biological processes that are being addressed, in order to enhance the products performance and provide a benefit to the patient. The quantity of drug required may be a function of how much is released over time to achieve the desired pharmacological effect. As drug is delivered locally from the device, the concentrations contained within the coating may be extremely low, particularly if the device itself is small, such as a DES. Total drug loading per device may be difficult to measure and would normally involve extraction of the drug from the coating in a suitable solvent (that can swell the coating and dissolve the drug) followed by quantification using techniques such as HPLC or other more specific techniques (Ranade et al., 2004). The solid state form of the drug is an important property to consider as this may influence its overall physical properties, effecting its dispersion within the coating when applied to the device. This can have major effects on mechanical properties of the coating, the drug release kinetics and its long-term stability. The drug may exhibit polymorphism and the ability to control its existence in crystalline to amorphous states may offer another mechanism of controlling its release; indeed, mixing a poorly water-soluble drug with the polymer coating itself may provide for beneficial drug crystal habit modification, resulting in altered release kinetics and enhanced bioavailability. The solid state form can be determined from films of polymer and drug that are subjected to a wide range of techniques such as differential scanning calorimetry (DSC), X-ray diffraction analysis (XRD) or polarized light microscopy. Further analysis can be conducted on the coatings themselves using methods such as atomic force microscopy (AFM), where amplitude-phase-distance (a-p-d) studies carrier out in Tapping Mode™ can yield information on crystalline material embedded within the coating matrix. Moreover, a modification of AFM known as Micro Thermal Analysis is able to perform DSC with the spatial resolution of the scanning probe microscopy, allowing for rapid localized thermal analysis, such as melting point determination of an identified drug crystallite (Price et al., 1999). Attenuated total reflectance Fourier-Transform infrared spectroscopy (ATR-FT-IR) is a technique that has gained popularity in recent years and not only enables the identification and quantification of pharmaceutical solids in coatings (Ding et al., 2009) but also allows quantitative analysis of mixtures of drug polymorphs (Helmy et al., 2003). This technique platform is now available coupled with microscopy and automated sample stage handling to provide mapping capability across a large area of the study sample (Lewis et al., 2004), (see for example the PerkinElmer® Spotlight Series FT-IR microscopes). Finally, techniques such as Dynamic Secondary Ion Mass Spectrometry (DSIMS) has been applied to the study of drug distribution within and release behaviour from polymer coatings (Verhoeven et al., 2004).

Coating analyses

There are a number of key properties of drug delivery coatings that must be considered when developing a drug-device combination product (Administration FaD, 2005):

Thickness	May determine the absolute dose of drug that can be contained within the carrier matrix and rate of drug elution; may be composed of several layers to modulate release kinetics
Uniformity	Ensures a consistent dose of drug is delivered per unit area of the coating; surface properties may also be important for biocompatibility. Continuous coatings may provide protection for the underlying substrate
Adhesion/cohesion	Ensures the coating maintains integrity over time, is durable to survive device deployment and in-use stresses without flaking or delamination. Adhesion to the substrate must be firm, cohesion within the coating or between different layers is fit for purpose
Composition	May provide mechanisms for drug interaction (charge, hydrophobic domains/phases, swelling-controlled release, biodegradation of the matrix). May determine mechanical properties (e.g., glass transition temperature (T_g)—effecting the ability of the polymer to flow and film-form, its elasticity, hardness and modulus)

There are a large number of techniques that can be used to characterize one or more of these important attributes, some of which are destructive and others which are non-destructive and potentially useful as quality control or in-line measuring tools (Fig. 11.3A and B). Of the non-destructive methods there are a number which can be usefully applied for the measurement of coating thickness, including: simple spectrophotometry or more complex spectroscopic ellipsometry, optical (white light) profilometry/interferometry, beam profile reflectometry, and confocal scanning laser or Raman microscopy. Each of these techniques has pros and cons depending upon required level of accuracy, depth resolution, multi-layer capability, translucence/refractive index properties, flat or curved geometry, amount of sample preparation, ease of use and cost considerations. Destructive techniques such as nanoindentation, focal depth indexing, stylus profilometry and some modes of AFM rely upon removal or displacement of an area of coating down to the substrate and some physical method of measuring the depth.

Case study: Coronary stent coatings

AFM has been used in conjunction with scanning electron microscopy (SEM) in the analysis of explanted polymer-coated coronary stent samples to determine the presence, thickness and mechanical properties of the coating several months post-implantation (Lewis et al., 2002a,b) and after release of drug into the artery (Lewis et al., 2004) (Fig. 11.3). The one caveat to this method however, is that it requires the underlying substrate to have different physical properties to the coating in order to distinguish when the substrate has been reached.

Scanning probe methods such as AFM can also provide information regarding the topography of the surface over a given area, enabling determinations of surface roughness and hence uniformity on the nano as well as microscale. Coupled with phase analysis, the instrument can

distinguish between different polymer phases or polymer and drug, as demonstrated for the styrene-isobutylene-styrene (SIBS) triblock copolymer coating on the Taxus® DES which has a microphase-separated structure (Ranade et al., 2004). These data were complimented with transmission electron microscopy (TEM) studies, where the different polymer phases were selectively stained using RuO_4 and imaged. AFM phase imaging of the SIBS coating has been used to demonstrate the presence of the drug preferentially associated with one of the polymer phases, and to monitor the effect of drug dissolution over time on the coating morphology. This instrumental method is used to continually scan an area with increasing levels of force applied to the scanning tip. This not only excavates an area of coating that can be used for depth profiling by cross-sectional analysis, as seen in Fig. 11.3C, but also provides an indication of the force that is required to completely remove the coating and hence a metric for investigating the influence of drug loading within the coating on its cohesive and adhesive properties. Combinations of techniques generate the most powerful data, as demonstrated by Belu et al in their analysis of coatings of poly(lactic-*co*-glycolic acid) (PLGA) mixed with rapamycin, using surface analysis by electron spectroscopy for chemical analysis (ESCA) and time-of-flight secondary ion mass spectrometry (TOF-SIMS) coupled with optical interferometry and confocal Raman microscopy (Belu et al., 2008).

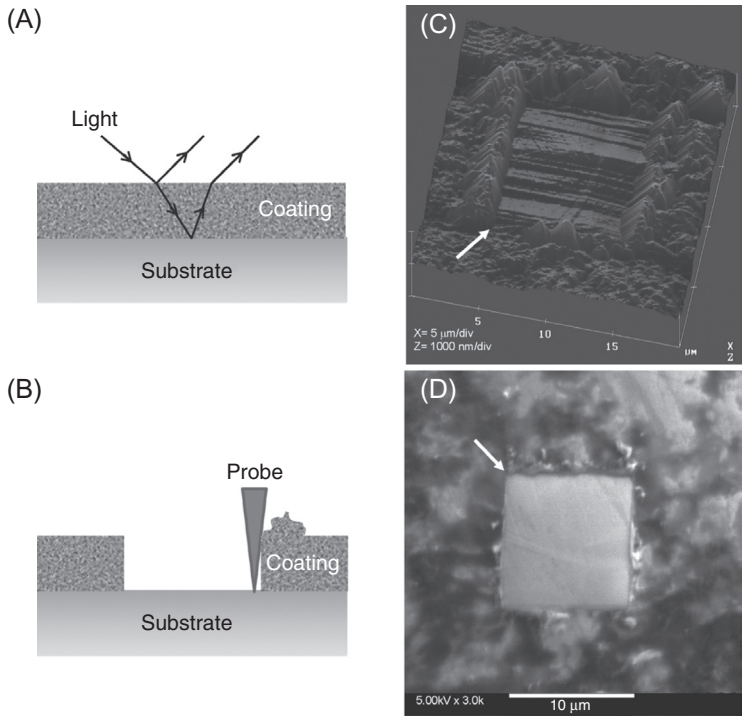


Fig. 11.3 Thin coating analysis. (A) Non-destructive techniques based upon reflected light (B) destructive techniques based upon probe penetration. (C) AFM surface image of an explanted polymer-coated stent where a 10 µm square of coating has been excavated by the AFM. (D) Corresponding SEM image of the excavated area.
Images (C) and (D) courtesy of Biocompatibles UK Ltd., Farnham, UK, www.biocompatibles.com.

More conventional mechanical property measurements can be obtained on cast polymer films (rather than the coated devices), using methods such as tensometry and dynamic mechanical analysis; but more relevant measures can be obtained using sensitive force-sensing probes that are now available (as outlined in Section 11.5.1.1), that can be applied to the direct analysis of drug delivery coatings themselves. Nano/micro scratch testing is possible using equipment such as the Nanovea® Mechanical Tester in Scratch Test mode, which applies loads in a controlled fashion to surfaces to study failures in thin coatings. The critical load at which failure occurs is both related to test-specific parameters, such as loading rate, scratch speed, indenter tip radius and material, as well as the test sample properties such as friction coefficient, thickness, hardness and roughness.

Part of the coating analysis needs to be conducted on the finished combination product to predict performance under clinically-relevant conditions. This type of testing can be focused on mimicking conditions for (i) the handling and administration of the product to the patients and (ii) the long-term in-use conditions over the product lifetime. The type of characterization test selected will therefore be based around the device and how it is used in practise. Some test equipment has been designed and is now commercially available to developers, specifically for the testing of particular devices. Bose offer a range of cardiovascular test instrumentation, including the Electroforce® 9210 DES test instrument which allows testing of 12 samples simultaneously, with pulsatile distension and particle capture technology to detect any shedding of the coating during simulated use over test periods up to 10 years simulated life time.

11.5.1.3 Drug stability, dosing and uniformity determination

Drug can be present throughout the matrix of the device itself (as for an antimicrobial agent in a bone cement, or chemotherapeutic in an embolisation bead), contained within a coating on the device or be deposited as a layer of neat drug on the surface of the device without the inclusion of carrier (as for various DES). When combined with the device in the final product, the manufacturer must show that the drug remains stable and is unchanged over time in its combined form. This will lead to shelf-life studies on the final product format (see Section 11.6.1). The practice of matrixing and bracketing may allow a range of different drug dose and device configurations to be tested without the additional significant time and cost of analysis of every iteration in between. Again, issues arise here if one of the configurations fails and a risk-based approach would recommend archiving of certain samples for recall and detailed re-analysis in the event of an unexpected failure. Drug dose and stability can be routinely measured using combinations of high through-put UV/Visible Spectrophotometry and HPLC methods with sufficient samples to demonstrate statistical significance in dose uniformity from batch to batch.

11.5.1.4 In vitro drug release characterization

A vitally important property for any drug-device combination product is the rate at which the drug is released. The elution of the drug can be controlled by a wide variety of different mechanisms ranging from simple dissolution from a surface based upon

drug solubility, to controlling its diffusion through a coating or the bulk material of the device itself. Whatever the mechanism of release, there needs to be an in vitro method to demonstrate the controlled and reproducible elution of drug from a combination product; this often forms part of a Quality Control release test. In developing such a method, a number of considerations should be made, including preparation of the device, the selection of the parameters for elution (temperature, pH, elution medium etc.), evaluation of the sink conditions for the drug, the choice of appropriate elution apparatus, identification of the analytical method for drug detection and quantification, sampling frequency and elution discrimination studies.

The US, European and Japanese Pharmacopeia outline a series of largely harmonized methods and apparatus for demonstration of drug dissolution and release. Although initially developed for the pharmaceutical industry to evaluate immediate release formulations, these methods have been modified to cater for sustained/delayed release modalities and for drug delivery via forms other than tablets, such as topical or transdermal delivery systems. These methods are also therefore being applied to the evaluation of the drug-release from combination products; the principle functions of the test being:

- To allow optimisation of the therapeutic efficacy of the product (demonstrate control over timing of the dose released).
- To ensure batch to batch reproducibility of release and hence a simple measure of product quality and physical consistency.
- Allow an estimation of the in vivo availability of the active, often by allowing an in vitro-in vivo correlation (IVIVC) to be made (once in vivo data are available) whereby the test can become a predictor of product performance.
- Allow comparison of performance between different products/formulations containing the same active agents and hence an estimation of "bioequivalence."

The various Pharmacopeia therefore present suitable requirements, test methods and apparatus to defined quality standards, to ensure the safety and effectiveness of medicines. Table 11.2 outlines the many chapters from both the United States Pharmacopeia (USP) and European Pharmacopeia (Ph.Eur.) relating to drug dissolution and release, which demonstrates the importance of this subject.

Apparatus for measuring drug elution

The methods and apparatus available for drug release evaluation are selected dependent upon the dosage forms of the drug (Fig. 11.4) (Karande and Yeole, 2006). USP apparatus 1–4 are concerned primarily with dissolution (mainly solid dosage forms), whereas USP apparatus 5–7 with drug release (mainly transdermal delivery (Zhou et al., 2007)). The vertical diffusion cell (Franz Cell) is currently being reviewed for inclusion for the testing of semi-solid dosage forms such as creams and gels. USP apparatus 1 (basket) and USP apparatus 2 (paddle) have been used for many years in the evaluation of solid oral dosage forms and validation of such techniques is well documented (Averell Frost, 2004). Drug is released into a specified volume of elution medium at 37°C over time, which is optionally circulated through a UV/Visible spectrophotometer flow-through cell which constantly monitors a selected wavelength

Table 11.2 Selection of US and European Pharmacopeia drug elution tests.

USP Chapter	Subject	Ph.Eur. Chapter	Subject
<711>	Dissolution	2.9.3	Dissolution test for solid dosage forms
<724>	Drug release	2.9.4	Dissolution test for transdermal patches
<1058>	Analytical instrument qualification	2.9.25	Dissolution test for medicated chewing gum
<1087>	Intrinsic dissolution	2.9.29	Intrinsic dissolution
<1088>	In vitro and in vivo evaluation of dosage forms	2.9.42	Dissolution test for lipophilic solid dosage forms (suppositories)
<1090>	In vivo bioequivalence guidances	2.9.43	Apparent dissolution (powders and granules plus various monographs on dosage forms)
<1092>	Dissolution procedure: development and validation		
<1224>	Validation of compendial procedures		
<1226>	Verification of compendial procedures		

characteristic of the drug in question. USP apparatus 2 could obviously be employed for the evaluation of a drug-device combination product, assuming the device was of such a dimension that it could be placed within the dissolution vessel and also that it was not damaged by the rotation of the paddle. Drug eluting stents (DES), for instance, have been evaluated by this technique; but often the amount of drug on the device is so small and the elution volume large such that several devices are required in order to release a detectable amount of drug. Hence, more commonly for expensive combination products such as DES is the use of one device immersed in a small volume of elution medium (typically 1–20 mL) from which aliquots are taken over time (being replaced by an equal volume of fresh medium) and then analyzed off-line using a sensitive analytical method such as HPLC (Ranade et al., 2004).

Although capable of demonstrating the consistency of release of the drug from the product, these types of elution methods are not representative of elution in vivo; here the device will be in contact with tissue into which the drug component must diffuse, as opposed to exposure to a large volume of liquid extraction medium. Hence, the relevance of the test for predicting release in vivo is much less. There have therefore been developments of methods that better relate to the in vivo situation, such as USP apparatus 4 that uses a flow-through cell into which the product can be placed and the flow of extraction medium around the product precisely controlled. Use of a USP or Ph.Eur. method is preferred where possible, as they are widely recognized and validated. Regulatory bodies however, will accept data generated from specially-designed and validated elution

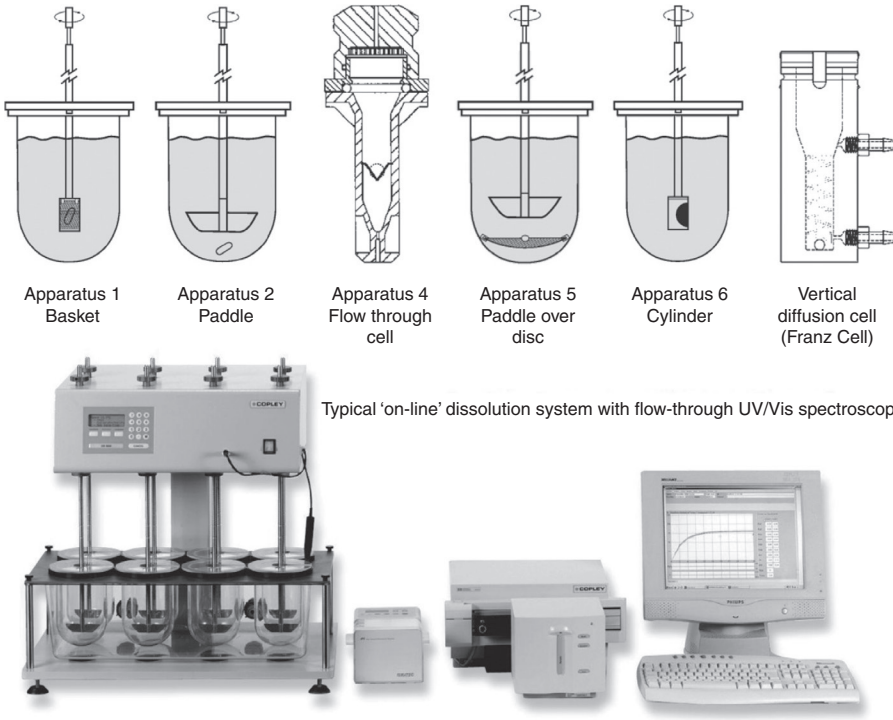


Fig. 11.4 Diagrammatic representation of the different USP Dissolution apparatuses and a typical on-line flow-through system.
Images and photograph courtesy of Copley Scientific Ltd., Nottingham, UK, www.copleyscientific.com.

tests if they attempt to measure drug release in a way more akin to the in vivo situation. For example, there have been reports of a modified USP apparatus 4 which attempts to make the test even more relevant, for instance by coating the interior of the cell with an alginate hydrogel into which a DES can be deployed and drug release measured into both extraction medium and hydrogel (Neubert et al., 2008). In the case of DEB, many have employed a so-called T-apparatus, which possesses a well into which the sample is placed and subsequently relies upon diffusion and convection processes to carry the drug into a circulating flow through circuit where its concentration can be monitored real-time (Borovac et al., 2006; Lewis et al., 2006a). Indeed, drug release data using this system have been subsequently correlated with human plasma drug level pharmacokinetic data, resulting in a Level A correlation, demonstrating that the technique is a good predictor of the expected plasma drug levels over the first 24 h of release from the combination product (Gonzalez et al., 2008) (see Section 11.5.3.4).

Drug elution media

An aspect of the method employed to monitor drug elution from the combination product is the choice of medium used to extract the drug. For simple quality control

purposes, a rapid extraction medium may be required to remove all drug from the device and allow quantification of the total dose. This may consist of a mixture of components and solvents into which the drug is readily solubilized. For drug release studies, it is preferred if the medium again mimics the in-use conditions as close as possible. For instance, elution into water is of limited value and as a minimum, phosphate buffered saline (PBS) would be recommended as it possesses a physiologically relevant concentration of ions. This is of particular importance where the drug release mechanism might be dependent upon ion-exchange processes (Gonzalez et al., 2008). Determination of the solubility of the drug in standard dissolution media (as per USP chapter <724>) is therefore important, which may be known already in the literature. Where the drug is particularly insoluble in aqueous media (as for some of the drugs used in DES such as rapamycin and paclitaxel) release into PBS is too slow to be of value in an elution method. Hence, in some cases additives such as surfactants like Tween®20 (Ranade et al., 2004), sodium dodecyl sulfate or Solutol® HS 15 (Chattaraj, 2007) are added to the elution medium to aid solubilization of especially hydrophobic compounds. Release into more complex biological media such as plasma has been reported but there are issues with the use of this medium past a few hours as it will start to degrade (Gonzalez et al., 2008).

Analysis and discrimination between drug elution data

Whilst the elution experiment may give an estimate of how much and how long drug is released from the device, it is also important for determining if release is consistent from one device to the next. Discrimination between elution runs is performed by carrying out the experiment several times on the same lot of device. Typically, dissolution data can be subjected to time point analysis where the percentage of drug release is reported at specified time points and the values obtained analyzed by conventional statistical methods such as one-way analysis of variance (ANOVA). Additionally, FDA have provided various guidance on the application of a mathematical treatment of the elution data (Administration FaD, 1997) that compares the profiles from the various runs and generates a number known as the similarity factor, f_2, defined by the following equation:

$$f_2 = 50 \log\left[1 + 1/n \sum_{t=1}^{n} \left(R_t - T_t\right)^2\right]^{-0.5} \times 100$$

where R_t and T_t are the percentage of drug dissolved at each time point. If an f_2 value between 50 and 100 is generated, the test suggests the elution profiles are identical; as the value becomes smaller, the results become more dissimilar. Different approaches to obtain similarity factor have been reported (Gohel et al., 2005) but the important objective is the use of a reproducible, robust and validated method.

11.5.2 Biocompatibility and toxicity testing

Biocompatibility testing for combination products is highly challenging and should be approached in a way that deals with the unique complexities of the combined entities. Safety assessment requires that both constituent components and combined product be tested, which can require vastly differing regulatory guidance. For a device drug

combination, the device should be tested according to ISO10993, Biological Evaluation of Medical Devices guidelines, whereas the drug component should be tested to ICH Guideline M3(R2) Maintenance of the ICH Guideline on Non-Clinical Safety for the Conduct of Human Clinical Trials for Pharmaceuticals. The combined product should then be tested using an appropriately considered mixture of the two guidances to incorporate intended use and clinical relevance. Consideration of the component parts is relevant here, as using previously approved constituents may reduce the testing burden, although prior data may well have been generated using a different route of administration and local dosing aspects may also not have been previously considered. A study if an potential interactions between the components is important as combination of the components could have adverse effects on key performance attributes, such as physical, mechanical and chemical properties, stability and efficacy.

It is expected that combination products that are intended for implantation within the body will undergo the full range of biocompatibility tests, as described throughout this book and defined under ISO10993 (for a detailed review of preclinical testing for drug-device combination products, see Chapter 15 (G. Clermont et al.) in reference (Lewis, 2010)). It may be necessary however, to undertake some of these tests on the device component only if the drug is to have a known influence on the outcome of the test; it is pointless running a cytotoxicity assay on a DEB containing an anticancer agent, if the drug is known to have a cytotoxic effect by a mechanism of action that will interfere with the test. Sample preparation is indeed an important consideration, as where ICH M3(R2) guidance for a pharmaceutical active may suggest testing at 50 times the clinical dose, this may be impossible or impractical for the combination product, particularly if the active is at low dose and is embedded within the device component (such as a coating in the case of many drug-eluting devices). Given the complexity of combination products, test protocols may include dose ranging studies, multiple doses in definitive studies (as have been performed for fast, medium and slow drug release modalities for Drug Eluting Stents for instance), with multiple test article applications and multiple sample collection points (to take into account the differing routes of administration that the combination product may require).

Toxicity evaluations for combination products involve aspects of both loco-regional and systemic effects and typically require in vivo studies of 1, 3 and 6 months minimum and possibly 12 months follow-up depending upon the expected duration of release of the drug component, its pharmacological effect and whether it already has a well-documented safety profile. Toxicity studies are acute and chronic in nature and may involve single and repeat dose evaluations. Wherever possible, studies should be performed to GLP or to the principles of GLP if the animal model is particularly specialized (for example, certain tumor models). This requires fully defined protocols, gross pathology of all important organs, full histopathological analysis and reporting with detailed blood chemistries where applicable.

11.5.3 Determining safety and efficacy in animal studies

Before undertaking studies in animals, background information should be gathered on the combination product to be tested, therapeutic target and mechanism of action,

proposed clinical setting and the intended effect in the target population. This should be supplemented by a review of the literature in order to place the test product within the landscape of other products with similar therapeutic intent. This will help in the study design, selection of the appropriate animal model and species and choice of primary and secondary end-points for the study. By defining the study purpose clearly, the correct type of study can be determined as these will clearly have different purposes:

- Feasibility study: a pilot or model development study
- Prototype evaluation study: to help guide the R&D process through design iteration
- Training study: to inform staff or intended users on how to administer/use the product
- Regulatory study: a key R&D decision making study conducted to the appropriate level of quality to enable use in regulatory submissions to support approval of the product

For combination products a phased approach of initial pilot study before entering more complex definitive studies is usually advised in order to gain experience in product administration and allow initial assessment of drug dosing for instance. Pivotal studies use established end-points and are designed to demonstrate the product works. Primary efficacy studies can be used to establish the principle actions or effects of the product, answering the question of how well it works, whereas secondary efficacy studies may evaluate the mode of action or effects not related to the intended therapeutic effect and may uncover additional adverse or beneficial affects of the product. Safety/Toxicity studies focus on the undesirable or adverse effects of the product at the desired dose and beyond to establish a window of safety where possible in order to define potentially harmful effects of the product.

For combination products that are medicated implants, long-term in vivo implantations described in Section 11.5.2 are designed to detect any undesired toxicity associated with the combination product that affects its ultimate safety. The objective of such studies is to provide evidence that the product can be used safely in humans and it is often required that the study be performed in such a way that that a safety margin is established. For instance, the concentration of a drug component may be selected so that it is in reality several-fold higher than that selected for initial human study. In some cases, an indication of product effectiveness may be inferred from the study in addition to the demonstration of safety. For example, the porcine coronary artery over-size balloon injury model is commonly used for long-term evaluation of DES and not only provides information on the local tissue response around the product in the vessel wall (local toxicity), but also due to the biological response to the arterial injury, a measure on how well the drug component can prevent smooth muscle proliferation in and around the stent (Karas et al., 1992; Steele et al., 1985). Moreover, the model demonstrates that the device component is also effective in carrying out its primary mode of action of holding open the coronary artery and maintaining good blood flow. More usually, the efficacy must be derived from an additional set of animal studies that are designed specifically to test the pharmacological activity of the drug component and/or the effectiveness of the device function.

11.5.3.1 Considerations when choosing animal models

In many cases the animal model will not be able to provide particularly relevant information regarding the efficacy of the product and indeed even in those models designed

to test effectiveness, the translation from animal to human pathophysiology is usually poor and provides only a weak indication that the product could work. Conversely, if the active under study has particular specificity for human biology, the inappropriate selection of an incompatible animal model to demonstrate efficacy could prematurely end the product development of a potentially effective therapy. Relevance of the model to the human condition is therefore an important consideration.

Case studies: Drug eluting intra-arterial devices

As mentioned in the Section 11.5.3, the porcine over-sized balloon injury model is commonly employed in the evaluation of DES; the biological response induced by the balloon injury in young pigs however, is only a surrogate at best for the complex and varied coronary artery disease (CAD) state manifested in largely elderly and diabetic patients. Other models such as the use of mini-pigs allows longer-term follow-up of the animals post-implantation, as standard farm swine grow too large to manipulate easily within theater; but these still do not possess CAD representative of humans. Some specialist animals have been bred specifically for such studies; hypercholesterolemic rabbits manifest CAD where there is more biological similarity in the disease processes with that of the human condition (Jang et al., 2009). These animals however, have smaller arteries and device placement in the heart is not usually possible; implants in the larger aortic or iliac arteries thus have the disadvantage that they do not have the organ-specific environment.

A similar situation exists for DEBs; a product designed for intra-arterial delivery into the hepatic artery to block blood flow to liver tumors and concomitant delivery of a chemotherapeutic agent. A relatively simple model of hepatic arterial embolisation is sufficient to demonstrate safety of the product (Taylor et al., 2007; Lewis et al., 2006b); enabling detailed pathological analysis of the effects of the combined arterial occlusion and local drug delivery, in addition to the ability to evaluate pharmacokinetics and hence estimate gross systemic exposure to the drug. This model does not however, provide any indication of whether the therapy would be suitable for treating a vascular solid tumor. A tumor model, preferably of hepatic origin or at least hypervascular in nature, is therefore desirable to demonstrate relevant efficacy for this product. Large animals such as sheep and pigs possess relevant arterial dimensions but there are currently no large animal liver tumor models. Again, the rabbit provides on approach with the well-characterized VX-2 tumor model which is hypervascular in nature and can be treated with a microcatheter and intra-arterial administration of the product. A tumor-bearing rat model of colorectal cancer to the liver has also been used in an embolisation setting (Eyol et al., 2008; Saenger et al., 2004). This model however, requires a complex surgical approach to allow infusion of the beads directly into the hepatic artery as access using microcatheters is impossible. Furthermore, the use of this model depends upon specially-made product that is small enough to pass through the tiny arteries of the rat and it must be recognized that the drug release kinetics of such a product could differ significantly from that being developed for human use. Therefore, as with all pre-clinical studies, caution must be taken when translating the results to the clinical setting.

Case study: Ophthalmic drug-device combinations

Ocular toxicity studies are not recommended in mice or rats and therefore often performed on rabbits, although care must be taken in the choice of breed as drug and metabolites are known to bind to melanin in certain types. Dogs or monkeys are often selected as the second species, both introducing significantly more cost. Test groups should be balanced across the sexes and typically

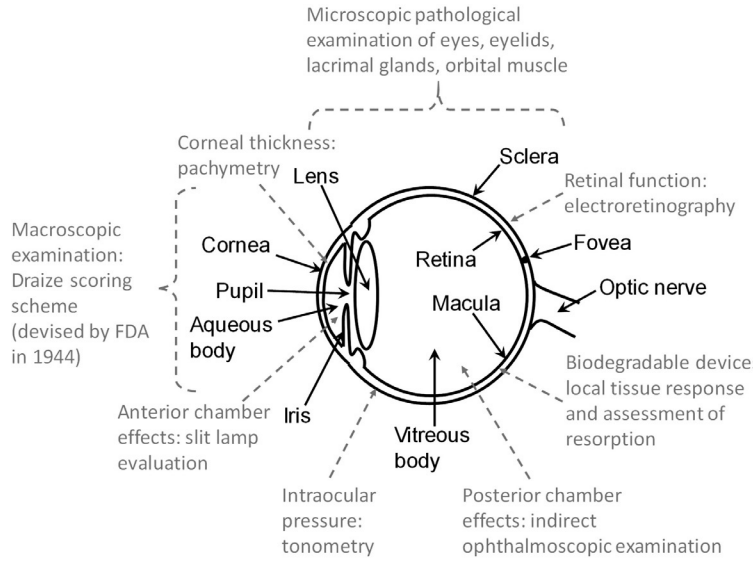


Fig. 11.5 Anatomy of the eye and recommended techniques for the evaluation of the ocular toxicity of an ophthalmic drug-device combination product.

consist of a control (carrier only), test article groups and a surgical sham if the device is implantable or untreated control if not. Assessment of the ocular toxicity of the product should consist of at a series of examinations as depicted in Fig. 11.5. It is recommended that the potential for systemic effects be checked by monitoring standard clinical measurements followed by necropsy and gross organ evaluation upon sacrifice (Short, 2008). Drug elution kinetics from the device should be performed in vivo (rabbits again are a typical model), with studies designed to collect by fluids (tears, aqueous and vitreous humor) and tissue (cornea, lens, retina) for determination of drug levels over time. Drug-device combination products cleared by FDA following these recommended tests include Retisert® (Fluocinolone acetonide) for treating chronic non-infectious uveitis in the posterior of the eye and Ozurdex® (Dexamethasone) for treating macular oedema.

11.5.3.2 Dose ranging/finding studies

Dose ranging/finding studies may be necessary to determine the appropriate dose that demonstrates both safety and efficacy. It may therefore be necessary to perform studies in a number of different animal models, as it may not be possible to obtain both safety and efficacy data in the same model (Section 11.5.3.1). Additionally, it is usually a requirement that tests are performed in more than one animal species. Such studies may involve both acute and repeat administration of the product where possible with subsequent assessment of the resulting toxicity. The aim is to determine the NOAEL (no observed adverse effect level) for the drug component and will mimic the dosing and frequency of administration that would be proposed for the clinical use of the product. Often it is desirable to demonstrate a safety factor to allow for interspecies differences such as tolerance to the drug or rate of its metabolism. An appropriate

safety margin may be determined based upon milligrams of drug per unit mass or surface area per day in order to translate to a human equivalent dose. These types of study are best carried out to GLP standards under approved protocols in recognized models where applicable. Fully documented autopsy reports are required with gross and histopathological examination of the relevant tissues/organs.

11.5.3.3 Pharmacokinetics and bioavailability

If the drug component is well-known and understood, it may not be necessary to conduct extensive absorption, distribution, metabolism and excretion (ADME) studies. By delivering a drug from a device however, it may be that the extent and duration of exposure to the active agent is altered compared to its normal route of administration. Drug pharmacokinetics (PK) therefore become an important aspect for the combination product and one of the key challenges in generating these data is often the relatively low doses of drug that are delivered. This would normally involve taking tissue local to the implant site, specific target organs and/or plasma samples, at various time points over a relevant period. The drug is extracted from the tissue (which in itself may require significant method development for the efficient removal of all of the drug) and then subjected to analysis using a sensitive analytical method such as HPLC or mass spectrometry. It may also be necessary to monitor the main known metabolites of the drug to gain an insight into whether there is a change in how the drug is processed within the body as a result of the change in delivery route. Depending upon the type of device under investigation, it may sometimes be difficult to separate the device from the tissue and hence the extraction process will remove drug that is both resident in the tissue and in the device itself. Even then, levels of drug may be so small that other techniques such as radiolabelling, or those that focus on the higher concentrations usually found local to the implant site may be employed (see Section 11.5.3.5). It may be possible to correlate the PK data by coupling it with the in vitro drug release data obtained as outlined in Section 11.5.1.4 to obtain an in vitro in vivo correlation, which is the subject of the following section.

11.5.3.4 In vitro-in vivo correlation (IVIVC)

In vitro-in vivo correlation (IVIVC) is defined as the relationship between in vitro dissolution and in vivo input rate. In many cases, however, dissolution is not the rate-limiting step in the elution of a drug. The FDA guidance on IVIVC provides general methods for establishment of IVIVC for oral formulations, but there is very little information available on the development of appropriate in vitro methods for IVIVC for non-oral forms (Administration FaD, 1997). The principles of this guidance can be applied to develop IVIVC for non-oral formulations (Ramana and Upoor, 2001) and although there have been a number of reports on in vitro methods correlation with non-oral drug release formulations, no standard procedure has been adopted (Cheung et al., 2004; Chidambaram and Burgess, 1999; Schliecker et al., 2004). In cases where a meaningful IVIVC can be developed, it can be used to predict the biological performance of a dosage in vivo. This may minimize the number of in vivo studies required

for dosage form development, allowing prediction of potentially toxic or ineffective formulations with both cost and ethical benefits (Leeson, 1995).

The IVIVC can be of three types:

- *Level A correlation:* Generally linear (although not always) with a point-to-point relationship between in vitro dissolution and some parameter derived from the in vivo data. Level A is the most useful and valuable type of correlation model and recommended where possible.
- *Level B correlation:* Uses statistical moment analysis which compares mean in vitro dissolution time with mean drug residence time or mean in vivo dissolution time.
- *Level C correlation:* This establishes a single point relationship between a particular pharmacokinetic parameter (such as C_{max} or AUC) and a dissolution/elution parameter (such as the time to reach *x*% dissolution/elution, or a dissolution/elution rate). This is considered the weakest of the correlations but may often be described in the context of multiple Level C correlations for a particular system.

A Level A correlation will aid in accelerating some aspects of the combination product development cycle by providing a predictive tool that links the in vitro release characteristics with the in vivo performance. In order to maximize the chances of obtaining a Level A correlation, time should be spent obtaining the optimal in vitro release data, with special attention to the relevance of the release apparatus, conditions and elution media (see Section 11.5.1.4).

11.5.3.5 Local drug distribution

Obtaining information on the local drug distribution in tissue delivered from a combination device is one of the major analytical challenges faced by the developers of the products. The difficulty in obtaining such data is somewhat related to the properties of the drug itself, in as much as how difficult it is to detect in low quantities. Naturally it is possible to adopt the methods used in the pharmaceutical industry when addressing questions such as biodistribution and fate of an entity. In such cases radiolabelling studies are commonly employed but these are invariably expensive in nature, as the drug has to be synthesized with a suitable non-labile radionuclide and the experiments carried out in facilities capable of handling radioactive compounds. Nevertheless, this technique remains a useful method for the analysis of combination products and one of the few that will enable an account of the whereabouts and fate of all of the drug post-delivery. Radiolabelled angiopeptin has been delivered from DES and autoradiographical images of histological sections were generated to demonstrate that the drug diffuses from the stent coating and into the vessel wall (Armstrong et al., 2002); moreover it showed the drug was still found in the surrounding tissue at least 28 days later (Lewis et al., 2001).

Where the drug is more easily detected, optical or spectrophotometric methods can be applied. DEB containing the highly fluorescent chemotherapeutic agent doxorubicin have been used in both animal and human studies where the tissue has been later recovered for analysis the drug distribution (Namur et al., 2010). Standard epifluorescence microscopy can generate spectacular images of drug penetration through the vessel wall and into the surrounding tissue over time (Lewis and Holden, 2011). Microspectrofluorimetry has been applied to histological sections of the processed

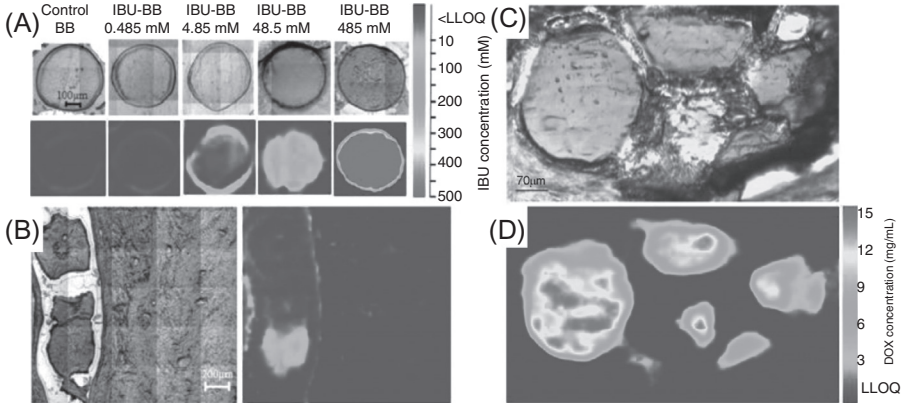


Fig. 11.6 (A—upper panel) Bead Block (BB) containing Ibuprofen (IBU) of different loadings, embedded and sectioned; (A—lower panel) corresponding FTIR image at 1512 cm^{-1} to visualize drug content in the beads. (B—left) Histological section of IBU-BB in a uterine vessel; (B—right) corresponding FTIR image at 1512 cm^{-1} showing IBU distribution. (C) Histological section of a vessel occluded with DEB containing Doxorubicin (DOX); (D) corresponding FTIR image at 988 cm^{-1} showing distribution of DOX.
Images courtesy of Dr. Julien Namur, Archimmed SARL, France.

tissues and by comparison with a series of controls derived from collagen imbedded phantoms, quantification of the drug levels in the tissue as a function of distance from the implant surface were obtained. Similar studies have also been performed using infrared microspectroscopy, which is useful where the drug is not inherently fluorescent and hence more difficult to detect (Namur et al., 2009). Coupled with the imaging capability of this technique it has been possible to generate images of drug distribution within, and quantification of in vivo release from, drug eluting implants containing drugs such as doxorubicin, irinotecan and ibuprofen (Fig. 11.6).

11.5.3.6 Antimicrobial efficacy (AME) testing

One area of combination product development that is becoming increasingly common is that of temporary or permanent device implants which deliver antimicrobial agents to tackle infections at the implant site. Such infections are not uncommon (typically 1–5%) and can be notoriously difficult to treat with systemic therapies. Often the only course of action is device removal which can be risky and is a considerable extra expense. By releasing a suitable antimicrobial agent at a therapeutic dose and over an appropriate time scale, any infection introduced at the time of the procedure or subsequently during wound-healing may be controlled more efficiently. It is therefore necessary during the development of such combination products, not only to have an understanding of the safety and pharmacokinetics but also to have evidence that the antimicrobial agent that has been selected is efficacious against the pathogens likely to be the cause of any potential infection. Table 11.3 outlines a selection of available methods suitable for the in vitro testing of antimicrobial combination products.

Table 11.3 Selection of AME test methods relevant to combination products.

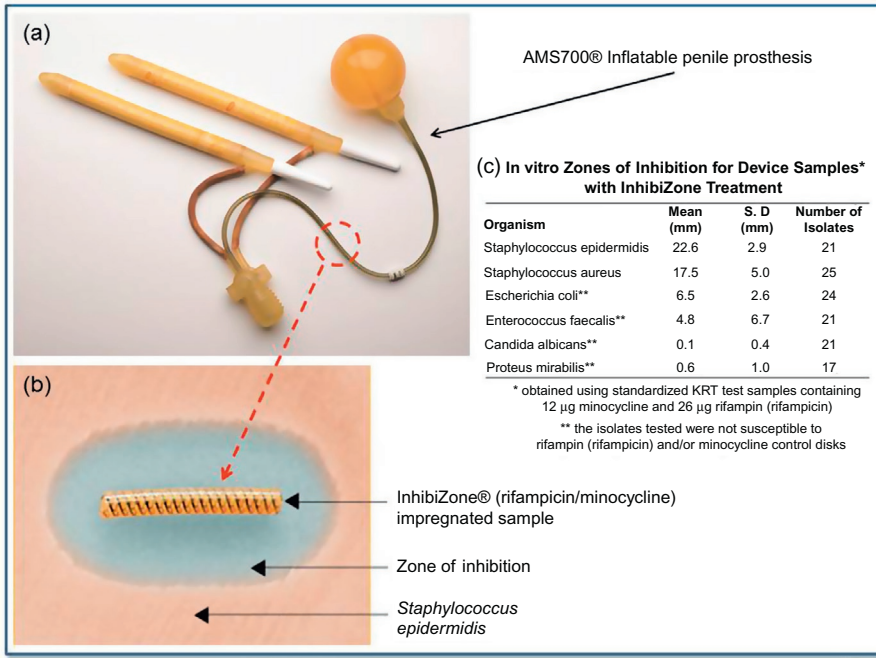
ASTM	Test
USP <51>	Antimicrobial effectiveness
USP <1227>	Validation of microbial recovery (neutralization validation)
ISO 22196	Antimicrobial activity, quantitative
ASTM E-2149	Dynamic contact (antibacterial, quantitative)
ASTM E-2180	Bound antibacterial activity
ASTM E-2315	Time-kill procedure
ASTM G-21	Antifungal (semi-quantitative)
Other	Zone of inhibition test

Effectiveness is typically measured by inoculated of the test product with known levels of a test organisms (dependent upon the most common cause of the infection targeted by the product, frequently *Escherichia coli* and *Staphylococcus aureus*). The reduction in microbe count is then measured over a 1 day (ISO 22196) to 28 day period (USP <51>). ASTM E-2180 is a specific test for antimicrobial agents contained within hydrophobic polymer materials (which may be the device itself or a coating material), which is contacted with a solution of a challenge organism such as *Pseudomonas aeruginosa* to mimic biofilm formation on the device. Percent reductions in the organism over time are calculated comparing the active test article versus a non-active control. ASTM E-2315 is applied when the product is intended to act over a prolonged time period and a kill time-course model is required. Zone of inhibition (or Kirby-Bauer disk diffusion assays) also provide a visual basis of measuring the potency of an antimicrobial that is released from a material by creating an area of no growth around the test article. This is illustrated in Fig. 11.7, with the AMS700® inflatable penile prosthesis. This device is impregnated with the antibacterial compounds rifampicin and minocycline, which clearly demonstrates an active zone of inhibition against organisms such as *Staphylococcus epidermidis* and *Staphylococcus aureus*.

Having demonstrated in vitro antimicrobial capability, regulatory bodies will usually also require an efficacy measure for the product in an in vivo infection model. Here, the devices can be implanted into subcutaneous pockets in a suitable animal, typically a rabbit, which is infused with a clinically-relevant strain of microorganism. After a specified time (which can be days to weeks), the implants are retrieved aseptically and the remaining viable organisms quantified by various techniques. This testing can be accompanied by other evaluation such as histological assessment or analysis of local tissue levels of the antimicrobial agent. All of this would be compared to non-active positive control implants.

11.5.4 Common inadequacies in preclinical testing

The testing outlined in this section should be performed in a thorough manner and to high quality standards for inclusion in regulatory submissions. Animal studies are best performed to GLP but it is recognized that some of the models employed are highly specialized and hence difficult or impossible to perform to such standards. In these



(c) **In vitro Zones of Inhibition for Device Samples* with InhibiZone Treatment**

Organism	Mean (mm)	S. D (mm)	Number of Isolates
Staphylococcus epidermidis	22.6	2.9	21
Staphylococcus aureus	17.5	5.0	25
Escherichia coli**	6.5	2.6	24
Enterococcus faecalis**	4.8	6.7	21
Candida albicans**	0.1	0.4	21
Proteus mirabilis**	0.6	1.0	17

* obtained using standardized KRT test samples containing 12 µg minocycline and 26 µg rifampin (rifampicin)

** the isolates tested were not susceptible to rifampin (rifampicin) and/or minocycline control disks

Fig. 11.7 (A) AMS700® Inflatable penile prosthesis. (B) Zone of inhibition experiment demonstrating antimicrobial efficacy of a section of tubing against *Staphylococcus epidermidis*. (C) Table of Zone of inhibition data from the device against various organisms. Photographs courtesy of American Medical Systems, Minnetonka, Minnesota, www.AmericanMedicalSystems.com.

cases, the studies should be performed to a pre-specified protocol, all experimental data should be recorded in detail and reported in appropriate appendices and the sites conducting the studies should have some degree of accreditation or be confident to be subject to audit of their data. Nevertheless, the complexity involved in pre-testing of combination products often results in inadequacies in the test data submitted for review. Portnoy and Koepke provided a relatively recent review of the most common inadequacies encountered in submissions (Portnoy and Koepke, 2005b), which have been reproduced in Table 11.4 as an illustration to the developers of such products where potential pitfalls may lie.

11.6 Aspects to consider in the manufacture of combination products

The combination product will be subjected to the detailed in vitro and in vivo characterization outlined in the previous sections throughout its development phase in order to optimize its performance. These types of tests are best carried out when the design of the product has been frozen and there are no other planned changes to the

Table 11.4 Common pre-clinical testing inadequacies in combination product submissions

Pre-clinical test	Common inadequacy
Bench evaluation	• Inadequate fatigue and corrosion testing • Inadequate analysis of surface modifications
Laboratory evaluation	• Inadequate testing of drug-coating integrity and durability • Inadequate particulate analysis (USP <788>) • Inadequate demonstration of chemical stability • Inadequate characterization of drug content and uniformity • Incomplete in vitro pharmacokinetic testing
CMC evaluation	• Poorly characterized CMC methodologies and specifications • Inadequate characterization of impurities • Unacceptable or poorly characterized toxicity data for leachables and/or residual solvents • Insufficient data on chemical effects of sterilization on finished product • Inadequate demonstration of product stability/shelf life
Animal studies	• Insufficient data to provide preliminary evidence of safety • Inadequate evaluation of clinically intended dose • Inadequate evaluation of overdosage • Unacceptably short-term duration of chronic follow-up • Inadequate evaluation of local-tissue toxicity • Inadequate evaluation of systemic toxicity • Inadequate or missing description of histopathology • Necropsy reports not included in submission (especially important for unexpected animal deaths)
Clinical evaluation	• Issues involving the duality, duration, and/or applicability of feasibility data • Omission of dose-ranging studies • Failure to fully consider pharmacological aspect of product • Failure to provide complete and comprehensive clinical results

CMC, chemistry, manufacturing and controls.
After Portnoy, S. and S. Koepke, Regulatory strategy: drug testing of combination products Medical Device and Diagnostic Industry, 2005a. June; Portnoy, S. and S. Koepke, Regulatory strategy: preclinical testing of combination products. Medical Device and Diagnostic Industry, 2005b. May, Copyright © 2005 Canon Communications LLC.

components, such as in concentrations of actives or carriers, inclusion of excipients, or alterations to the processes used in fabrication that could introduce new or increased levels of residuals. Such changes could affect the performance characteristics of the product and ultimately its safety and effectiveness profile. If changes are required in order to facilitate scale-up to manufacturing capacity, additional bridging studies may be required to show the combination product performance remains unchanged.

11.6.1 Shelf-life

Shelf-life is one important parameter that should be considered at an early stage and defines the duration and conditions over which the required performance of

the combination product is achieved. The stability of the final combined product ultimately defines how long it can be held in inventory or on the customer's shelf from the date of manufacture before it exceeds its shelf-life and must be discarded. But shelf-life is relevant to the product throughout the development cycle, with each stage having a unique set of durations and conditions that are governed by specific design requirements and controls. One should however, consider what is required to be suitable for the intended use of the product, such as the need for sterility, the delivered volume, reliability, degradation of the active component; and capture this as a design input. The focus should be on the safety and efficacy, risk and critical quality attributes of the product. The design inputs and outputs should also record the factors that could potentially affect the product performance over time, including processing, sterilization, shipping and transit (including cold chain if required), storage conditions.

A shelf-life should be identified considering user needs, supply chain constraints and any drug component expiration. Design inputs should be established for the key stages and design outputs developed together with a verification plan. Guidance on this provided by FDA in 21 CFR 211.137 on expiration dating and 21 CFR 166 on stability testing. CGMP Requirements for Combination Products January 2017 clarifies the expiration assignment for combination products and is clear that device and drug product constituents may have different expiration dates (such as for kits), for which case the expiration is set to the earliest of the components. Shelf-life testing can be performed under accelerated conditions for a quicker indication of stability; often this is not the recommended approach, as the components of the product may be more susceptible to degradation at the elevated temperatures used in the test and regulatory authorities do eventually require real-time aging data under ambient conditions. The product should be tested against its release specification to ensure it continues to perform as predicted; this may involve a range of different tests, often composed of those described throughout this chapter that have been used in the characterization of the product throughout its development. A typical measure for a drug component would involve extracting the drug from the product by a validated method and subjecting it to a sensitive analytical technique such as HPLC to ensure the drug has not undergone any degradation. The kinetics of drug release should also remain unchanged and will need to be tested to a pre-determined percentage of total dose (normally at least 80% elution) and compared to the reference in the product specification using the similarity method outlined in Section 11.5.1.4. As with shelf-life, shipping studies also require the same type of evaluation of the product after it has been sent to and back from a specified destination in order to simulate transport conditions. Typically, humidity and temperature would be monitored during shipping and attention would also be paid to the integrity of the product packaging.

11.6.2 Sterilization

Combination products are usually supplied sterile and may be sterilized by conventional terminal sterilization techniques such as ethylene oxide (EtO), steam

sterilization or gamma irradiation. It may be that certain components of the product could be destroyed by the sterilization step and it is necessary to undertake the required level of characterization testing to demonstrate that the performance characteristics of the product remain unchanged post-sterilization. Sterilization by EtO is often a batch process and it must be shown that there is no cross-contamination from the residuals of a previous sterilization run or different product being processes at the same time. Again, analytical methods like HPLC coupled with techniques that provide structural information such as nuclear magnetic resonance (NMR) spectroscopy can be useful in determining if a drug is altered by the sterilization process. If this transpires to be the case, the method of manufacture may have to be altered in order to produce the combination product under aseptic conditions, which requires an additional level of control and testing to ensure sterility is maintained in the end product.

11.7 Clinical studies for combination products

11.7.1 Combination product retrieval studies

Although now somewhat beyond the scope of this chapter, the pre-clinical data generated in support of the safety and efficacy of a combination product will be used in a submission to approve clinical evaluation of the investigational product. Many of the techniques and methods developed and applied at the pre-clinical stage may also be of utility in the clinical phase; for instance, methods developed for PK analyses or techniques for evaluating the product if it becomes available post-explant of the device, or tissue in which it is sited. For example, the AFM technique described in Section 11.5.1.2 was developed and used to determine the thickness of a stent coating, both post-processing and also after in vivo implantation. During a routine clinical procedure on a patient, a coated stent was captured by accident and a section of it retrieved for analysis (Lewis et al., 2002a). The same AFM techniques were applied to demonstrate that the coating remained intact and maintained its original thickness and mechanical properties, which was a useful validation of the pre-clinical data.

In some cases it is possible for the removal of the product to be planned as part of specific treatment regime. DEB loaded with doxorubicin were used in a study to treat patients with hepatocellular carcinoma (HCC), in order to keep their disease under control whilst they were on a liver transplant list. Once a donor liver became available, the patient had the transplant and the diseased liver (containing the product) was subsequently analyzed in order to elucidate the whereabouts of the beads and the distribution of the drug in the tissue. A number of patients were treated with bridging periods from just 1 day to 36 days, which allowed a time-course of drug distribution within the liver to be recorded. Once again, this study validated the pre-clinical findings, which demonstrated that the drug levels and time-course generated in a non-tumor bearing porcine liver embolisation model were very similar to that observed in human patients with HCC (Namur et al., 2011).

11.7.2 Brief consideration for combination product clinical trial design

Clinical evaluation of a combination product needs to be designed to determine its performance, not only in terms of safety, but also that there is a clinical benefit that arises as a consequence of the combination. The degree of clinical evidence required will likely depend upon the nature of the product and the label claims being pursued. When planning a clinical trial, a cost-benefit assessment must therefore be made to determine financial viability of conducting such a study. For example, if the combination product contains an antibacterial compound and the manufacturer wishes to claim reduced rates of infection, yet the infection rate for the standard device is already relatively low, the patient numbers required to demonstrate a statistically significant decrease in infection rate in a randomized controlled study are likely to be so high as to make such an investment untenable. Hence, the manufacturer may wish to consider an alternative indication to the efficacy claims, which may be attainable based upon pre-clinical data alone.

Unlike clinical studies for medical devices, the combination product may require several phased clinical trials similar to those conducted for medicinal products. This may involve a dose-escalation aspect to determine or evaluate a maximum (tolerated) dose, as was the case for the first studies of DEBs used in the treatment of liver cancer (Poon et al., 2007; Varela et al., 2007). In these phase I/II type studies, a first cohort was treated with a fixed volume of beads, in which the dose of drug was escalated over groups of three patients to a maximum dose (based upon the current maximum recommended systemic dose). Once it was ascertained that this dose was safe with no dose-limiting toxicities, the second cohort were then treated with this maximum dose. This study also involved a pharmacokinetic analysis to determine systemic drug levels released from the device, which could be later compared with the pharmacokinetic data generated from pre-clinical models and correlated with in vitro drug release data (Gonzalez et al., 2008). Similarly, for many of the first-in-man DES studies, a number of trials were performed with either different doses of drug on the stent (Gershlick et al., 2004), or different release modalities (e.g., fast, moderate or slow release formulations) (Silber et al., 2009; Colombo et al., 2003), reflecting that the period over which the drug is eluted from the device will be critical depending upon the underlying biological response that is being addressed.

These types of ranging studies are then usually followed-up with bigger studies under intended-use conditions (Cohen et al., 2004). An approval for a combination product will require a randomized study that proves statistically that the product is at least equivalent or better (by whatever metric deemed central to the function of the product) compared to the existing standard of care; these sorts of trials are usually multicentre in nature to achieve the necessary recruitment of the hundreds of patients that will be required to drive a positive statistical outcome that is adequately powered. The applicant and regulatory body will negotiate/discuss the details of the clinical trial design that is required in order to support the marketing authorisation application for the product. Active control arms are preferred to those based upon a placebo, not least for ethical reasons. The advantage of a combination product that is the first of its type

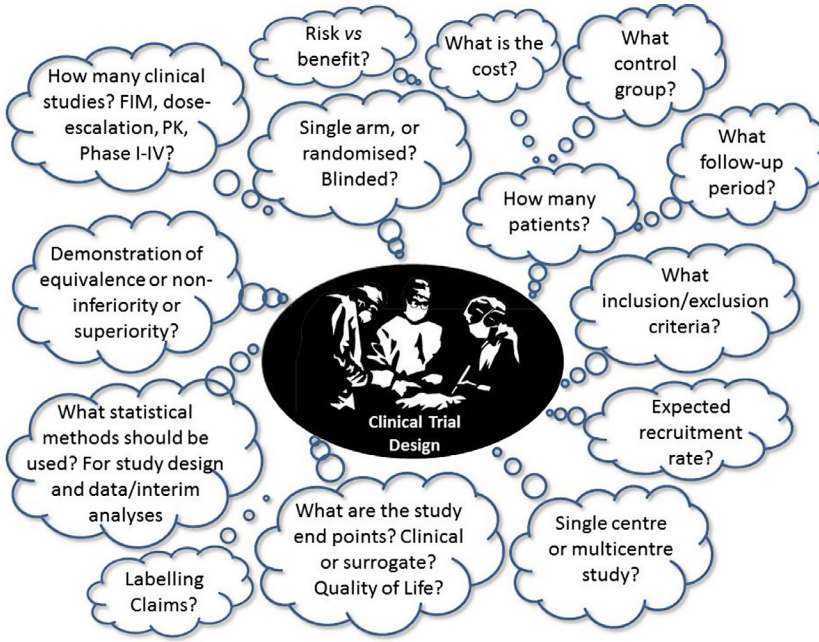


Fig. 11.8 Key considerations when designing a clinical trial programme for a combination product.

is that it may be randomized against current therapy as a control where it may require fewer patients to power a delta in performance. Early studies for DES randomized against bare metal stents and easily demonstrated superiority (Moses et al., 2003); nowadays, new DES designs are compared against currently approved products and trials geared towards proving substantial equivalence in performance (Chevalier et al., 2007). The intention of this section was to provide a flavor of what is involved in clinical evaluation of combination products and to demonstrate that it is a complex matter which encompasses a wide variety of considerations (see Fig. 11.8), however, the details of these considerations are way beyond the scope of this chapter.

11.8 Future outlook for combination products and their evaluation

Combination products offer potential for a step-change in product performance and subsequently significant clinical benefits to the patient. As more novel combination products are being developed and brought to market, there must also be the development of characterization methods that are both appropriately designed to reflect the function of the product and also analytically sensitive enough to detect the low levels of active compounds present. Truly representative in vivo models that provide valuable information on both safety and effectiveness aspects of the combination

product remain one of the biggest challenges. Regulatory bodies are experiencing an increase in combination product submissions and are responding by creating departments and guidelines to help manufacturers through the approval process. However, the presence of both device and drug elements is, and will continue to cause some confusion and debate, as those offices responsible for reviewing these elements approach product approvals from different perspectives. The successful product developers will be those that engage with the regulators at an early stage to define the requirements for an approval, enabling them to design and employ the most suitable characterization methods in order to demonstrate their product is ready for human use.

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