UNIVERSIDADE D
COIMBRA

Ana Rita Machado Cardoso

Relatórios de Estágio sob orientação do Dr. João Pimentel e de João Ferreira e Monografia intitulada "Hydrogel 3D models for mimicking the prostate cancer microenvironment" sob orientação do Professor Doutor Luís Maria Bimbo, referentes à Unidade Curricular "Estágio", apresentados à Faculdade de Farmácia da Universidade de Coimbra, para apreciação na prestação de provas públicas de Mestrado Integrado em Ciências Farmacêuticas.

Setembro de 2023

UNIVERSIDADE D
COIMBRA

Ana Rita Machado Cardoso

Relatórios de Estágio sob orientação do Dr. João Pimentel e de João Ferreira e Monografia intitulada "Hydrogel 3D models for mimicking the prostate cancer microenvironment" sob orientação do Professor Doutor Luís Maria Bimbo, referentes à Unidade Curricular "Estágio", apresentados à Faculdade de Farmácia da Universidade de Coimbra, para apreciação na prestação de provas públicas de Mestrado Integrado em Ciências Farmacêuticas.

Setembro de 2023

Eu, Ana Rita Machado Cardoso, estudante do Mestrado Integrado em Ciências Farmacêuticas, com o n.º 2018287127, declaro assumir toda a responsabilidade pelo conteúdo do Documento Relatórios de Estágio e Monografia intitulada "Hydrogel 3D models for mimicking the prostate cancer microenvironment" apresentado à Faculdade de Farmácia da Universidade de Coimbra, no âmbito da unidade de Estágio Curricular.

Mais declaro que este Documento é um trabalho original e que toda e qualquer afirmação ou expressão, por mim utilizada, está referenciada na Bibliografia, segundo os critérios bibliográficos legalmente estabelecidos, salvaguardando sempre os Direitos de Autor, à exceção das minhas opiniões pessoais.

Coimbra, 1 de setembro de 2023,

Ana Rita Machado Cardoso

(Ana Rita Machado Cardoso)

Agradecimentos

À minha mãe, que sempre me motivou, apoiou incondicionalmente e proporcionou a realização deste sonho.

Aos meus irmãos, por terem sido um pilar e por toda a força que me transmitiram.

Ao meu pai, que foi motivação e sei que estaria orgulhoso.

Ao Professor Doutor Luís Maria Bimbo por toda a atenção, disponibilidade e orientação na elaboração desta monografia.

A toda a equipa da Farmácia Adriana por toda a orientação, confiança depositada em mim, amizade e ensinamentos.

A toda a equipa de Garantia da Qualidade da Farmalabor, por todo o apoio e disponibilidade na realização do estágio.

A todos os meus amigos que fizeram de Coimbra casa.

Obrigada!

Índice

Parte III - Monografia - "Hydrogel 3D models for mimicking the prostate cancer microenvironment"

Parte I

Relatório de Estágio em Farmácia Comunitária

Farmácia Adriana

Orientado por Dr. João Pimentel

Abreviaturas

ANF	Associação Nacional de Farmácias
COE	Contraceção Oral de Emergência
FA	Farmácia Adriana
FC	Farmácia Comunitária
MNSRM	Medicamento não sujeito a receita médica
PVP	Preço de Venda ao Público
SWOT	*Strengths, Weaknesses, Opportunities and Threats*

1. Introdução

O conteúdo programático do Mestrado Integrado em Ciências Farmacêuticas culmina na realização do Estágio Curricular. Esta é a etapa final que tem o objetivo de integrar e colocar à prova as competências adquiridas no decorrer do curso.

A Farmácia Comunitária (FC) destaca-se das restantes áreas devido à sua ampla cobertura geográfica, onde, em muitas regiões, é a única estrutura de saúde disponível.[1] Como resultado, o farmacêutico é frequentemente o primeiro profissional de saúde a ser consultado. As competências destes profissionais permitem estabelecer uma relação próxima, acessível e de confiança com a população.

A realização do Estágio Curricular em FC proporciona uma profunda compreensão da importância da intervenção farmacêutica na comunidade, demonstrando o papel do farmacêutico como agente de saúde pública e especialista do medicamento.

O presente relatório documenta e descreve o estágio realizado na Farmácia Adriana (FA), sob orientação do Dr. João Pimentel. O período de estágio compreendeu as datas de 9 de janeiro a 31 de abril de 2023, e será abordado mediante uma análise SWOT (*Strengths, Weaknesses, Opportunities and Threats)*. Por fim, serão apresentados cinco casos práticos presenciados durante o estágio que evidenciam a aplicação dos conhecimentos adquiridos ao longo do curso.

2. Farmácia Adriana

A Farmácia Adriana (FA) está localizada no coração da cidade de Coimbra, na emblemática Praça da República. Caracteriza-se por ser uma das farmácias mais antigas da cidade, contando com mais de 100 anos de existência. A localização central, aliada à proximidade de comércio, restauração e ao Polo I da Universidade de Coimbra fazem desta praça um ponto de referência e passagem para muitos estudantes, turistas e residentes. Esta localização privilegiada reflete a diversidade de utentes que frequentam esta farmácia.

A sua equipa é composta por três elementos: o Dr. João Pimentel, Diretor Técnico, a Dra. Ângela Mota e a Sra. Adélia Guerra.

O seu horário de funcionamento é: de segunda-feira a sexta-feira das 8:30 às 20:30 e sábados das 9:00 às 20:00, com pausa para almoço das 13:00 às 14:00. Esta conta com uma *Pharm Machine* que oferece uma variedade de produtos garantindo que as necessidades dos utentes são atendidas mesmo fora do horário de funcionamento. A FA é afiliada à rede de Farmácias Portuguesas da Associação Nacional de Farmácias (ANF), e apresenta dois

fornecedores com duas entregas diárias, de modo a suprir a necessidade de medicamentos e produtos.

3. Análise SWOT

Figura 1 - Análise SWOT do estágio realizado em FC.

3.1 Pontos Fortes

Equipa Técnica

Na FA encontrei uma excelente equipa composta por profissionais qualificados, experientes e dedicados. A minha integração foi facilitada pela calorosa receção e disponibilidade dos colaboradores da FA. O apoio mútuo, o respeito e uma comunicação eficiente reiteraram a equipa ao longo do estágio. É importante destacar que a procura pela melhoria contínua e a excelência no atendimento são os principais compromissos destes trabalhadores. Assim, como desafio, surgiu a rubrica "1 minuto na Farmácia Adriana" onde semanalmente eram publicados vídeos relacionados a temas de saúde, com o objetivo de contribuir para a literacia em saúde dos utentes.

O dinamismo da equipa proporcionou-me uma experiência enriquecedora. Ao longo do meu estágio, a equipa estimulou-me a assumir responsabilidades e a desafiar-me constantemente, contribuindo para o meu crescimento profissional.

Aprendizagem Gradual e Personalizada

Durante a realização do meu estágio não houve um plano previamente definido, mas sim, uma atribuição progressiva de tarefas. Numa fase inicial foram discutidas as atividades a serem desenvolvidas, as competências a adquirir e os objetivos a serem alcançados. Comecei

por ser integrada no trabalho de *backoffice*, que foi importante para me familiarizar com os produtos da farmácia, locais de armazenamento destes e toda a logística da FA. Experiência que permitiu, subsequentemente, a realização de atendimentos de forma mais eficiente. As tarefas designadas nesta etapa consistiram na verificação de *stocks*, validades, e na receção e entrada de encomendas. O Sifarma 2000® foi o software utilizado, que me permitiu proceder a estas tarefas, bem como efetuar encomendas instantâneas, encomendas diretas, fazer o controlo de medicamentos psicotrópicos, processar devoluções e gerir *stocks*. Sendo que já estava familiarizada com estes processos devido a experiências anteriores em estágios de verão em FC nos quais trabalhei inteiramente no *backoffice*, fui alocada para o atendimento ao público relativamente cedo. No decorrer do atendimento ao público tive oportunidade de dispensar medicamentos sujeitos a receita médica, informando o utente sobre a posologia, eventuais medidas não farmacológicas que poderia adotar e esclarecendo dúvidas que este pudesse ter. Além disso, explorei o aconselhamento de produtos, incluindo medicamentos não sujeitos a receita médica (MNSRM), produtos de dermofarmácia e cosmética, e suplementos alimentares.

Considero que a falta de um plano de estágio previamente definido, foi ultrapassada pela implementação de uma aprendizagem gradual e personalizada.

Autonomia

O atendimento ao utente é o pilar do exercício farmacêutico em FC. Desde cedo, fui encorajada a interagir com o utente e a exercer esta tarefa de forma autónoma. Esta autonomia permitiu-me aplicar na prática conhecimentos teóricos e explorar habilidades de comunicação. Além disso, o facto de ter de tomar decisões autónomas levou-me a procurar aprimorar os meus conhecimentos, contribuindo para uma melhor aprendizagem. A meu ver, a confiança depositada em mim incentivou a minha autoconfiança na prática.

Diversidade de Serviços

A FA dispõe de diversos serviços como: a medição de parâmetros bioquímicos, a medição da pressão arterial, a determinação de peso e índice de massa corporal, a administração de injetáveis e vacinas, e a preparação individualizada da medicação. Esta é ainda aderente do programa de troca de seringas, um serviço de intervenção em saúde pública que visa a prevenção de propagação de doenças transmissíveis em utilizadores de drogas injetáveis. Neste procede-se à troca de seringas usadas por um kit que contém seringas, toalhetes desinfetantes, preservativos, ampolas de água bidestilada, filtros, recipientes para preparação

da substância e carteiras de ácido cítrico. A sua localização próxima de áreas frequentadas por utilizadores traduz-se na grande procura deste serviço.

A diversidade de serviços disponibilizados, nos quais tive a oportunidade de participar, foi fundamental para ampliar a minha visão da importância da intervenção do farmacêutico comunitário na prevenção, manutenção e monitorização de patologias.

3.2 Pontos Fracos

Uso do Sifarma 2000®

Como anteriormente referido a FA está associada à rede Farmácias Portuguesas da ANF, assim sendo dispõe dos serviços associados a este grupo, nomeadamente o Sifarma®. O software Sifarma® é um sistema de gestão, amplamente utilizado em FC, com inúmeras funcionalidades.[2] No entanto, durante o meu período de estágio o Módulo de Atendimento não foi utilizado. A utilização do Sifarma 2000®, com que não estava familiarizada, foi preferida em detrimento do Módulo de Atendimento, o que se traduziu numa dificuldade, pois é mais antigo e menos intuitivo. Destaco esta preferência como uma fraqueza, uma vez que a utilização do Módulo de Atendimento é o sistema preferido durante as aulas e aquele que prevalece na maioria das farmácias. Considero que a atualização para este sistema mais moderno seria benéfico para otimizar a experiência do atendimento tanto para o utente como para o profissional.

Dificuldade de associação entre denominação comum internacional e marca

Durante o plano curricular de MICF, a abordagem à denominação dos medicamentos é, principalmente, feita pelo seu princípio ativo. No entanto, apesar de as receitas serem prescritas com a Denominação Comum Internacional (DCI), os utentes, por vezes, pretendem apenas a dispensa parcial da receita, e solicitam os medicamentos pelo nome comercial. Inicialmente, isto identificou-se um desafio, pois desconhecia o nome comercial de muitos medicamentos, e foi necessário recorrer a um colega ou ao sistema informático para conseguir cumprir o atendimento. Gradualmente, consegui contornar esta dificuldade e associar os nomes comerciais ao seu DCI. No entanto, não deixo de destacar esta lacuna como um ponto fraco, uma vez que, aquando do atendimento passa a ideia de desconhecimento total do medicamento, descredibilizando a minha aprendizagem.

Erros de *stock*

A gestão eficiente do *stock* é essencial para evitar a falta de produtos. Durante o meu período de estágio, deparei-me com diversos erros de *stock*. A discrepância entre o número

de produtos apresentados no sistema e o *stock* real da farmácia levaram a constrangimentos durante o atendimento ao público. Em diversas ocasiões, informei o utente que o produto desejado estava disponível, mas acabava por confirmar o contrário, resultando em reações negativas e muito desagrado.

3.3 Oportunidades

Formações

Durante o meu estágio fui introduzida a diversas plataformas de formações, tal como a "Academia Perrigo" e "*Learning to Care*". Estas plataformas abrangem uma ampla variedade de temas, como cuidados da pele, suplementação e aconselhamento de MNSRM. O método de entrega de aprendizagem e avaliação de conhecimento nas plataformas é idêntico. Este consiste numa apresentação, seguida de um questionário. Estas formações permitiram-me consolidar e complementar conhecimentos obtidos nas unidades curriculares, além de me proporcionarem um maior entendimento de diversos produtos disponíveis na FA e, assim, realizar um aconselhamento mais informado. Destaco estas formações por representarem um método diferente de aquisição de conhecimentos, e por possibilitarem o contacto com diferentes e novos produtos no mercado de forma flexível e acessível.

Diversidade de utentes

Como referido anteriormente, a localização central, próxima de locais turísticos, restauração e comércio, resulta numa grande variedade de utentes ocasionais. Esta diversidade de utentes inclui diferentes faixas etárias, diferentes nacionalidades e, consequentemente, diferentes patologias. Esta multiplicidade de utentes permitiu-me adquirir habilidades e conhecimentos para atender às necessidades individuais de cada utente. Além disso, a oportunidade de interagir com diversas nacionalidades permitiu-me explorar um pouco a noção de FC noutros países e aperfeiçoar o meu inglês. A heterogeneidade proporcionou atendimentos variados que me ajudaram a aprimorar o meu sentido crítico e a saber como atuar em diversas situações.

3.4 Ameaças

Medicamentos Esgotados

Ao longo do meu período de estágio deparei-me com uma situação recorrente de medicamentos esgotados ou rateados, durante semanas ou até meses. Alguns destes sem alternativa terapêutica. Esta impossibilidade de dispensa afetou significativamente a terapêutica

de muitos utentes, deixando-os sem acesso a medicamentos necessários. Alguns utentes demonstraram frustração e incompreensão quanto a esta situação, uma vez que não compreendiam a razão pela qual estes medicamentos não estavam disponíveis na farmácia. Esta falta de compreensão representou dificuldades no momento do atendimento.

Locais de venda de medicamentos não sujeitos a receita médica

Os MNSRM podem ser vendidos fora das farmácias, em locais que cumpram certos requisitos.[3] Estes locais de venda e o seu fácil acesso em grandes superfícies comerciais ou através da *internet*, representam uma ameaça económica para FCs. Normalmente, estes têm a capacidade de praticar preços de venda ao público (PVP) menores do que os das FCs. Criando, assim, uma situação insustentável para as farmácias que não conseguem fazer frente a PVPs tão reduzidos. Adicionalmente, estes locais são desprovidos de profissionais tão capacitados para o aconselhamento como aqueles que se encontram em FC. Estes profissionais possuem um menor conhecimento científico constituindo um perigo para a saúde do utente.

Alguns utentes, após requisitarem aconselhamento na FA, acabaram por adquirir os MNSRM nestes locais, confrontando-nos ainda com a disparidade dos valores. A possibilidade de adquirir estes produtos noutros locais desvaloriza o exercício farmacêutico e causa constrangimentos durante o atendimento.

Proximidade a outra farmácia

A presença de outra farmácia localizada a cerca de vinte metros da FA resulta numa divisão dos utentes, tanto ocasionais como potenciais utentes recorrentes. Esta proximidade a uma concorrente cria uma opção adicional para os utentes, o que representa um desafio, pois há competição direta. Isto resulta numa diminuição do número de atendimentos realizados na FA. A meu ver, esta proximidade impactou diretamente a diversidade e quantidade de casos observados, o que, por sua vez, limitou as oportunidades de prática.

5. Casos Práticos

Caso Prático1

Uma mulher de 35 anos dirigiu-se à FA porque apresentava desconforto e prurido na região genital. Referiu a existência de um corrimento vaginal espesso e de cor branca, com um odor forte. Expliquei que segundo o descrito estaria com uma candidíase, uma infeção fúngica vaginal, causada pela *Candida albicans*, uma infeção comum que resulta de um desequilíbrio e que pode ser causada por diversos fatores. Após análise procedi ao aconselhamento de um creme vaginal com aplicador contendo 10mg/mg de clotrimazol, devendo aplicar uma vez por

dia ao deitar durante 6 dias. Incentivei o uso de roupa de algodão evitando roupa apertada, bem como evitar usar pensos ou tampões higiénicos com perfume e proceder a uma boa higiene, usando um gel de higiene íntima sem sabão e com agentes calmantes. Por último indiquei que caso os sintomas não desaparecessem deveria consultar um médico.

Caso Prático 2

Um utente do sexo masculino com idade compreendida entre os 20 e 25 anos dirige-se à FA, indicando que nos últimos dias teria sentido muita comichão nas mãos e que necessitava de algo para a aliviar. Ao examinar, observei a presença de vermelhidão e pequenas vesículas exsudativas, com um aspeto descamativo. Após conversar com o utente e obter mais informações, este mencionou uma possível associação ao uso de produtos de limpeza. Com base nestas informações suspeitei que se tratasse de uma dermatite de contacto.

Aconselhei o uso de um creme corticosteroide, especificamente a hidrocortisona 10mg/g, devendo ser aplicada duas a três vezes por dia, nas áreas afetadas. Ressaltei que esta terapêutica não deve ser usada durante mais de 7 dias, e caso não exista melhora após este período deverá consultar um médico. Além disso recomendei o uso de um creme emoliente para ajudar na hidratação da pele. Expliquei a importância da adoção de alguns comportamentos preventivos, como lavar a pele com produtos de limpeza suave, sem sabão ou fragâncias, e secar bem as mãos após lavagem. Orientei o utente a utilizar luvas quando fosse necessário manipular os produtos de limpeza de modo a evitar o contacto direto com a pele.

Caso Prático 3

Utente do sexo feminino com cerca de 20 anos dirige-se à FA na intenção de adquirir uma "pílula do dia seguinte". Demonstrava estar preocupada com a possibilidade de uma gravidez indesejada.

Abordei a utente e verifiquei que se tratava de uma situação onde houve falha do método barreira durante uma relação sexual, o único método de contraceção utilizado. De modo a verificar se esta toma poderia ser realizada perguntei qual a fase do ciclo menstrual em que se encontrava e a data da última menstruação para excluir a possibilidade de se encontrar grávida. Excluí ainda a possível concomitância de substâncias que pudessem ter interação com a contraceção oral de emergência (COE) ou a existência de problemas de saúde que impossibilitassem a sua toma. Com a confirmação que a relação tinha ocorrido há menos de 24 horas, procedi à dispensa de levonorgestrel, Postinor®, a COE mais adequada neste caso. Durante o aconselhamento, expliquei que a COE não deve ser utilizada como método

contracetivo regular, que este age através do bloqueio temporário da ovulação e que este método não é 100% eficaz na prevenção da gravidez. Além disso, referi alguns possíveis efeitos secundários e alertei para um possível atraso na menstruação seguinte. Aconselhei a realização de um teste de gravidez caso o atraso ultrapassa-se 5 dias. Por fim, referi que caso a utente vomite até três horas após a toma, esta deve ser repetida. Realcei a ausência de proteção por parte da COE contra doenças sexualmente transmissíveis.

Aconselhei, ainda, a jovem a considerar uma consulta de planeamento familiar para discutir opções contracetivas regulares mais adequadas ao seu estilo de vida e necessidades, de modo a evitar a recorrência de uma situação desta.

Caso Prático 4

Um utente com idade compreendida entre os 30 e os 35 anos, que está de férias em Portugal há cerca de cinco dias, procura aconselhamento para conseguir ter uma noite de sono descansada. Relata sentir-se extremamente cansado e ter dificuldade em dormir. Após algumas questões, constato que o motivo que o mantém acordado durante a noite são episódios de tosse. Ele refere que além da tosse, que por vezes apresenta expetoração, também apresenta congestão nasal sem sinais de secreção nasal.

Após descartar causas alérgicas, COVID-19 e outras comorbilidades, expliquei-lhe que o melhor a fazer era tratar a causa subjacente do seu cansaço. Para alívio da tosse, aconselhei o uso de uma solução oral, o Bronchodual®, um medicamento tradicional à base de plantas que contém extrato seco de tomilho e extrato líquido de raiz de alteia. Com a toma de 15mL a cada 3 a 4 horas, 4 vezes por dia.

Para aliviar a congestão nasal, sugeri o uso de um descongestionante nasal em spray, o Rinerge®, que contém 0,5mg/ml de cloridrato de oximetazolina. Expliquei que deve ser aplicado duas vezes por dia, de preferência de manhã e à noite, com 2 ou 3 nebulizações, em cada narina. Expus ainda algumas medidas não farmacológicas que poderia adotar como lavar as fossas nasais com soluções de água do mar ou soro fisiológico e aumentar a ingestão de líquidos. Aconselhei consultar um médico caso os sintomas persistissem ou piorassem.

Caso Prático 5

Um utente do sexo masculino com cerca de 45 anos deslocou-se à FA e solicitou algo para tratar as hemorroidas. De modo a obter mais informação para avaliar a gravidade da situação, questionei se este era um problema frequente e se estava associado a obstipação e dor, ao qual o utente me responde positivamente queixando-se que as suas fezes são duras. Com esta informação, aconselhei um creme retal, Procto-Glyvenol®, para aplicar

na zona afetada. Enfatizei, ainda, as alterações que poderia adotar na sua alimentação e no seu estilo de vida, como a introdução de fibras a ingestão de mais água, com vista a facilitar o processo de defecação.

Considerações Finais

A minha inserção na realidade profissional revelou-se essencial para o meu crescimento enquanto futura farmacêutica e profissional de saúde. A realização deste estágio, fomentou a minha perspetiva da atual realidade da FC. Percebi que um bom profissional tem de procurar estar sempre atualizado primando por uma formação contínua e multidisciplinar. As capacidades de comunicação e sensibilização são, também, fulcrais para um bom exercício da profissão.

A realização deste estágio em FC permitiu-me consolidar e aplicar conhecimentos teóricos, e adquirir novas competências. Estou confiante que esta experiência me preparou durante estes quatro meses. O apoio e a busca por tornar a minha experiência enriquecedora foram fundamentais para o sucesso desta etapa.

Referências Bibliográficas

1. **A Farmácia Comunitária - Farmácia Comunitária - Áreas Profissionais - Ordem dos Farmacêuticos** - [Consult. 1 set. 2023]. Disponível em: https://www.ordemfarmaceuticos.pt/pt/areas-profissionais/farmacia-comunitaria/a-farmacia-comunitaria

2. **Sifarma** - [Consult. 1 set. 2023]. Disponível em: https://www.glintt.com/pt/o-que-fazemos/ofertas/SoftwareSolutions/Paginas/Sifarma.aspx

3. **Locais de venda MNSRM - INFARMED, I.P.** - [Consult. 1 set. 2023]. Disponível em: https://www.infarmed.pt/web/infarmed/entidades/licenciamentos/locais-de-venda-de-medicamentos-nao-sujeitos-a-receita-medica-lvmnsrm

Parte II

Relatório de Estágio em Indústria Farmacêutica

Farmalabor

Orientado por João Ferreira

Abreviaturas

EC	Estágio Curricular
FFUC	Faculdade de Farmácia da Universidade de Coimbra
GQ	Garantia da Qualidade
IF	Indústria Farmacêutica
MICF	Mestrado Integrado em Ciências Farmacêuticas
SWOT	*Strengths, Weaknesses, Opportunities and Threats*

1. Introdução

O Mestrado Integrado em Ciências Farmacêuticas (MICF) apresenta uma abordagem multidisciplinar com o objetivo de preparar profissionais competentes em toda a atividade farmacêutica. No 5º ano de MICF, a Faculdade de Farmácia da Universidade de Coimbra (FFUC) permite que, para além do estágio obrigatório em farmácia comunitária, seja possível a realização de um estágio noutra área do medicamento. Esta oportunidade para os estudantes de MICF abrange qualquer área do ciclo do medicamento. Estas são investigação, clínica, tecnologia, indústria e regulamentação. A minha curiosidade pela Indústria Farmacêutica traduziu-se na escolha desta área para a realização do Estágio Curricular (EC).

O presente relatório documenta e descreve o estágio realizado na Farmalabor, no departamento da Garantia da Qualidade (GQ), sob orientação do Dr. João Ferreira. O período de estágio compreendeu as datas de 2 de maio a 28 de julho de 2023, e será abordado mediante uma análise SWOT (*Strengths, Weaknesses, Opportunities and Threats)*. Serão descritos os Pontos Fortes, os Pontos Fracos, as Oportunidades e as Ameaças que afetaram a minha experiência.

2. Farmalabor – Grupo Medinfar

Fundado em 1970, o Grupo Medinfar tem sede em Venda Nova, na Amadora. Conta com uma Unidade de Produção Industrial, denominada Farmalabor em Condeixa-a-Nova. Esta empresa farmacêutica exerce funções há cerca de cinco décadas. Atua em áreas como investigação, desenvolvimento e fabrico de produtos farmacêuticos, cosméticos e suplementos alimentares. É líder em Portugal na categoria de Saúde do Consumidor e Dermatologia, e a 3ª maior no top 5 de empresas portuguesas. A sua presença estende-se por mais de 40 países.[1]

A Unidade Industrial de Produção é uma parte fundamental ao lado de outras entidades como a Medinfar Consumer Health, Mendifar Maroc, Medinfar Sorológico e Medinfar Laboratório.[2] Para além de fabricar os produtos das suas marcas próprias, a Farmalabor também atua como *Contract Manufacturing Organization*. Esta empresa possui capacidade de produção de diversas formas farmacêuticas. Esta compromete-se a altos padrões de qualidade e rigor na produção dos seus produtos farmacêuticos. A sua abordagem integra sustentabilidade ambiental e a segurança dos seus trabalhadores. Isto reflete a rigorosa adesão às Boas Práticas de Fabrico e de Laboratório. Além disso, a empresa é certificada em áreas de Qualidade, Saúde e Segurança no Trabalho e Ambiente, atendendo às Normas ISO 9001, ISO 45001 e ISO 14001.[3]

3. Análise SWOT

Figura 2 - Análise SWOT do estágio realizado em IF.

3.1 Pontos Fortes

Integração na Equipa

A equipa do departamento de GQ caracteriza-se por ser um grupo jovem, proativo e empenhado no bom desempenho das suas tarefas. A Farmalabor prioriza a boa comunicação e o bom ambiente. No primeiro dia, fui recebida de forma calorosa e, desde logo, aceite como membro da equipa. Os colaboradores do departamento sempre procuraram contribuir para o meu conhecimento da área, e demonstraram disponibilidade para esclarecer qualquer dúvida.

Destaco a integração como ponto forte devido ao acolhimento praticado pela equipa. Revelaram confiança no meu trabalho e concederam autonomia para realizar as minhas funções. A dinâmica de colaboração da equipa de GQ contribuiu positivamente para a minha experiência de estágio.

Atividades Executadas

Como iniciação do meu percurso, assisti a uma apresentação sobre a empresa e a sua história. Participei ainda em algumas formações relevantes para o decorrer do estágio. Ao iniciar as minhas atividades, fui integrada na área de Gestão Documental. Neste contexto, executei uma variedade de tarefas, incluindo a revisão de documentação. Além disso, tive a oportunidade de colaborar na execução de funções em outras áreas da GQ, como do Controlo de Alterações. Considerando que a GQ tem um grande contacto com outras secções, surgiu a preocupação por parte da equipa, de me familiarizar com estas. Desta forma, visitei todos os departamentos onde outros colaboradores detalharam os processos, a

dinâmica e o funcionamento de cada. Estas visitas incluíram as secções de: produção de sólidos orais, a produção de líquidos, pastosos e semi-sólidos não estéreis, o controlo de qualidade, o controlo em processo e o armazém.

Ao interagir com outros departamentos, tive a oportunidade de obter uma visão mais abrangente da IF e todas as suas possíveis saídas profissionais.

Desenvolvimento de Diversas Competências

Ao longo da execução da maioria das minhas tarefas, foi necessário o uso do Excel® e do Word®. Inicialmente, a minha proficiência nestes programas era básica, mas ao longo do estágio, desenvolvi esta habilidade significativamente. Graças à necessidade do seu uso, hoje possuo um maior e mais eficaz aproveitamento destas ferramentas.

O uso predominante do inglês na IF e a natureza internacional das parecerias do Grupo Medinfar, fez-me aprimorar os meus conhecimentos neste idioma. Foi necessário aprender termos técnicos e praticar o inglês de forma intensiva devido à necessidade do seu uso diário.

O facto de diversas tarefas serem atribuídas simultaneamente levou a uma necessidade de organização e gestão de tempo, criando uma grande oportunidade de progresso destas capacidades.

Em suma, o período de estágio revelou-se fundamental para aperfeiçoar algumas *soft skills*, que serão certamente significativas, pois muitas vezes pertencem a requisitos para a prática profissional.

3.2 Pontos Fracos

Ausência de um Plano de Estágio

Um dos aspetos que apresentou falhas no decorrer desta experiência foi a ausência de um plano de estágio. Por vezes, esta ausência resultou em períodos de inatividade nos quais fiquei à espera de atribuição de novas tarefas. No contexto de indústria, este planeamento é fundamental, devido à elevada carga de trabalho dos elementos da equipa de GQ. Em certas ocasiões, instalou-se uma sensação de monotonia e falta de estimulação. Seria benéfico implementar um plano de estágio para uma gestão de tempo mais eficaz. Este permitiria ainda, uma maior autonomia e produtividade, sem requisitar tarefas constantemente. Desta forma, a minha contribuição para a Farmalabor seria mais eficiente, e não teria a necessidade de adicionar uma preocupação aos colaboradores.

3.3 Oportunidades

Nova saída profissional

A diversidade de conhecimentos do farmacêutico permite a sua inserção em diversos cargos. Ao longo do meu percurso académico, sempre tive curiosidade em trabalhar na área da IF. Ao estagiar na Farmalabor, tive a oportunidade de experienciar o quotidiano de uma empresa dedicada à produção do medicamento.

Este estágio permitiu-me desenvolver múltiplas competências e, deste modo, abrir caminhos para o meu crescimento em IF. Esta foi uma etapa significativa no meu percurso académico, reforçando o meu interesse em IF como trajetória profissional.

3.4 Ameaças

Duração do Estágio

O meu EC em IF teve uma duração mínima de 420 horas. Considero que este tempo foi insuficiente, especialmente porque alguns conceitos e procedimentos não foram amplamente explorados. Muitas tarefas que desempenhei foram partes integrantes de processos complexos. No entanto, teria sido valioso poder visualizar o progresso e a conclusão de algumas delas. A duração limitada do estágio impactou a minha capacidade de absorver completamente a essência destes processos. Uma maior duração permitiria colaborar em projetos mais detalhados e extensos, tendo uma experiência mais abrangente das tarefas em que estava envolvida.

Considerações Finais

A oportunidade dos estudantes da FFUC de realizarem um EC em diversas áreas representa um acréscimo significativo à sua formação académica. Esta vantagem proporciona uma visão mais aprofundada das possíveis trajetórias profissionais após a conclusão do curso.

A IF é uma área abrangente que aporta diferentes possibilidades de carreira e explora diversas competências. A minha experiência nesta área foi altamente benéfica tanto para o meu crescimento profissional como pessoal. Permitiu-me consolidar conhecimentos adquiridos ao longo de cinco anos e explorar uma área na qual tinha bastante interesse. Passei a compreender melhor a importância de uma cultura colaborativa e o impacto positivo que esta tem na motivação e desempenho de uma equipa. Estagiar na Farmalabor foi um privilégio. Este estágio ilustrou a natureza multidisciplinar do papel do farmacêutico.

Agradeço a toda a equipa da GQ. Especialmente, ao João Ferreira que me motivou e orientou neste percurso. Após esta experiência, sinto-me realizada e preparada para o meu futuro como Mestre em Ciências Farmacêuticas.

Referências Bibliográficas

1. **Quem Somos - Sobre Nós | Grupo Medinfar** - [Consult. 23 ago. 2023]. Disponível em: https://www.medinfar.pt/pt/sobre-nos/quem-somos

2. **Estrutura do Grupo - Sobre Nós | Grupo Medinfar** - [Consult. 23 ago. 2023]. Disponível em: https://www.medinfar.pt/pt/sobre-nos/estrutura-do-grupo

3. **Certificação - Indústria | Grupo Medinfar** - [Consult. 23 ago. 2023]. Disponível em: https://www.medinfar.pt/pt/industria/certificacao

Parte III

Monografia

"Hydrogel 3D models for mimicking the prostate cancer microenvironment"

Orientado pelo Professor Doutor Luís Maria Bimbo

Abstract

Prostate cancer is one of the most diagnosed malignancies in men, and an established leading cause of death. This condition is characterised by a significant heterogeneity and pathophysiological complexity. Its tumour microenvironment plays a crucial role in disease progression and therapeutic effectiveness, with its influence on therapy resistance well-established.

3D hydrogel models provide a platform to investigate prostate cancer in a more physiologically relevant context. These models offer insights into key aspects of the pathology's biology, including cellular plasticity, interactions between cancer cells and the surrounding environment, metastasis, and therapeutic response. By faithfully replicating the intricate features of the tumour microenvironment, 3D hydrogel models have the potential to increase our understanding of the disease and contribute to therapy development.

This Monograph addresses the complexity of prostate cancer and emphasises the need for using 3D hydrogel models to replicate its microenvironment. The available 3D hydrogel models are detailed, along with various characteristics of the hydrogels.

Keywords: Prostate Cancer; 3D Models; Hydrogel; Differentiation; Dedifferentiation; Microenvironment; Mimic; Resistance; Metastatic.

Resumo

O cancro da próstata é uma das doenças oncológicas mais diagnosticada nos homens, constituindo uma das suas principais causas de morte. Esta condição é caracterizada por uma grande heterogeneidade e complexidade fisiopatológica. O seu microambiente tumoral desempenha um papel crucial na progressão da doença e na eficácia terapêutica, assim como na influência da resistência às terapêuticas convencionais.

Os modelos 3D em hidrogel oferecem uma plataforma privilegiada para investigar o cancro da próstata num contexto similar ao fisiológico. Assim, estes modelos proporcionam uma perceção de aspetos biológicos fundamentais desta patologia, incluindo a plasticidade celular, as interações entre as células cancerígenas e o ambiente circundante, a metastização e a resposta terapêutica. Ao reproduzir fielmente as complexas características do microambiente tumoral, os modelos 3D em hidrogel têm o potencial de melhorar a compreensão da doença e contribuir para o desenvolvimento de novas e mais eficazes terapêuticas.

Esta Monografia aborda a complexidade do cancro da próstata e destaca a necessidade do uso de modelos 3D em hidrogel para replicar o seu microambiente. Os modelos 3D em hidrogel disponíveis são aqui detalhados, assim como diversas características dos mesmos.

Palavras-chave: Cancro da Próstata; Modelos 3D; Hidrogel; Diferenciação; Dediferenciação; Microambiente; Mimetizar; Resistência; Metastização.

Abbreviations

ACT	Alpha-1-antichymotrypsin
ADT	Androgen Deprivation Therapy
AFS	Anterior Fibromuscular Stroma
APCs	Antigen-presenting Cells
AR	Androgen Receptor
ART	Adjuvant Radiation Therapy
AS	Active Surveillance
BPH	Benign Prostatic Hyperplasia
BT	Brachytherapy
CAF	Cancer-associated Fibroblasts
CRPC	Castration Resistant Prostate Cancer
CSCs	Cancer Stem Cells
CTC	Circulating Tumour Cells
CZ	Central Zone
DHT	Dihydrotestosterone
DRE	Digital Rectal Examination
ECM	Extracellular Matrix
EMT	Epithelial-mesenchymal transition
HIFU	High-intensity-focused Ultrasound
IMRT	Intensity-modulated Radiation Therapy
LDR	Low-dose-rate
LH	Luteinizing hormone
LHRH	Luteinizing hormone releasing hormone
MDSCs	Myeloid-derived suppressor cells
MET	Mesenchymal-epithelial transition
mpMRI	Multiparametric magnetic resonance imaging scanning
PCa	Prostate Cancer
PET/CT	Positron Emission Tomography/Computed Tomography
PIN	Prostatic Intraepithelial Neoplasia
PSA	Prostatic-Specific Antigen
PSMA	Prostate-Specific Membrane Antigen
PZ	Peripheral Zone
TAM	Tumour-associated macrophages

TGF-β	Tumour growth factor-beta
TME	Tumour Microenvironment
TNM	Tumour Nodes Metastasis
TP	Transperineal
TR	Transrectal
TRUS	Transrectal Ultrasound
TZ	Transition Zone
SRT	Salvage Radiation Therapy
UGE	Urogenital Sinus Epithelia
UGM	Urogenital Sinus Mesenchyme
UGS	Endodermal Urogenital Sinus
USA	United States of America
VMAT	Volumetric Arc Radiation Therapy

1. Introduction

As life expectancy continues to rise, an increasing number of individuals experience cancer. Prostate Cancer (PCa) affects millions of men all over the world and is the most frequently diagnosed cancer in men in Portugal.[1] In addition, it is the second most frequent cancer and the fifth leading cause of cancer-related deaths in men worldwide.[2] It is projected that in 2023, 288300 new cases of PCa and 34700 deaths by this malignancy are expected in the United States of America (USA) alone.[2] The projections also indicate that mortality from this malignancy will double from 2018 to 2040.[3] This carries a burden for healthcare systems, in the USA alone, that is estimated that be approximately one million prostate biopsies are conducted annually, just this results in an average cost of $1694 per patient.[4]

PCa has heterogenous nature, due to its intra-tumoral heterogeneity and cellular plasticity.[5] It presents different rates of incidence and mortality across different regions due to the screening programs, diagnosis strategies applied, and the influence of well-established risk factors.[6] Age, ethnicity, genetic factors, and family history are variables to consider. Studies have shown that the risk of PCa tends to rise after the age of 50 for Caucasian men and 45 for African men or those with a family history of PCa. African-American men have a higher predisposition for PCa and in a more aggressive form, observations proposed that it can be associated with several genetic variations.[3]

1.1 The gland – Anatomy and Histology

The prostate gland is a structure of the male reproductive system, an accessory reproductive organ that facilitates the process of reproduction, is pyramid-shaped and it is situated below the bladder in front of the rectum and surrounding the urethra (Figure 1). The base of the prostate contacts with the seminal vesicles, one each side. The size of this exocrine gland varies with age, but a normal prostate weight is about 15-20 g. Its main function is to produce and secrete fluids that protect and nourish the sperm while increasing the volume of semen to facilitate propulsion through the urethra.[7] The anterior aspect of it consists entirely of fibromuscular, while the posterior part and the region surrounding the ejaculatory ducts as they enter the urethra in both side of the verumontanum, is glandular.[8] Consequently, this gland can be histologically divided into three glandular zones, known as McNeal zones: the central zone (CZ), the transition zone (TZ) and peripheral zone (PZ). The CZ corresponds to 25% of the prostate, has a conical structure with ramifications from the verumontanum (where the ejaculatory and prostatic ducts penetrate the posterior wall of the prostatic urethra) to the prostatic base and surrounding the ejaculatory ducts. Normally it is not the

site of origin of any disease process, but only disease-related area when secondarily affected by cancer. The TZ, is adjacent to the prostatic urethra, composed of ducts that extend laterally from the urethral wall and curve in an anteromedial direction, and only 5% of the gland, being imperceptible in majority of young men. Is the exclusive location of the development of benign prostatic hyperplasia (BPH), an enlargement of the gland because of a benign nodular proliferation of the tissue. PZ, it is the outer portion, with ducts extending in a posterolateral and anterior direction from the urethra, starting at the verumontanum to the prostatic apex and relates to 70% of the prostate and has the highest incidence of carcinomas. [9 10 11] The part of the prostatic urethra that is not included in PZ, the anterior zone, is covered by a fibromuscular stroma. This anterior fibromuscular stroma (AFS) is a non-glandular region that constitutes the fourth zone of the prostate. It is like a barrier and occupies a significant section of the anteromedial prostatic tissue from the apex to the base. The lower muscular fibres of the AFS are supported by striated muscles of the urethral sphincter, while the upper fibres are supported by fibres of the detrusor muscle of the urinary bladder, all mixed with connective tissue. It provides protection for prostatic urethra and glandular zones from adjacent structures since it frequently merges with the prostatic glandular tissue anterior and lateral to the urethra. All of those are encapsulated by a fibroelastic tissue, a thin sheet of an internal layer of smooth muscle and an external collagenous membrane, where are combined prostatic plexuses of veins and nerves. This capsule differs considerably from area to area as a result this cannot be considered a well-defined anatomical component. It is known that this layer is thinner in the posterior side, typically fused with the Denonvillers' fascia, a compact collagenous membrane that directly lays with the wall of the rectum muscle. [11]

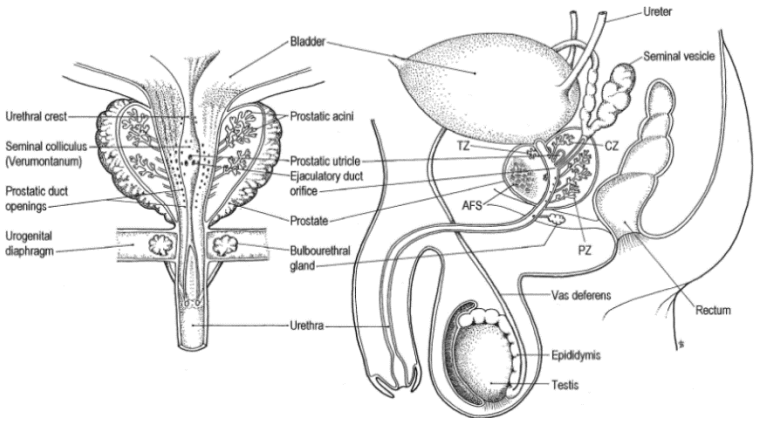

Figure 1 - Location of the prostate and anatomical regions. AFS: anterior fibromuscular stroma; CZ: central zone; PZ: peripheral zone; TZ: transition zone.[8]

The epithelium and stroma of the human prostate often exhibit a diverse range of changes. The stroma is fibromuscular with smooth muscle cells mixed with elastic fibres that surround the glandular tissue. The glandular epithelium is composed of acini and ducts covered by three types of cells: luminal, basal, and neuroendocrine. All these types of cells have the same progenitor, the multipotent basal cells that differentiate during the prostate organogenesis. In most cases the acini have a swelling to papillary look. This papillary structure is more visible in the central zone. The luminal cells are characterised by their columnar shape, light eosinophilic cytoplasm and a round nuclei located close to the base of the cell. These are specialised cells responsible for secreting various substances into the lumen, contributing to the formation of seminal fluid. One notable product of those that they produce is prostate-specific antigen (PSA), and luminal cells show strong positives for PSA immunohistochemistry tests. The number of basal cells can vary within the glands of individuals. These cells are positioned adjacent to the basement membrane along with the luminal cells and display ovoid nuclei and inconspicuous cytoplasm. The neuroendocrine cells are rare, they exhibit both endocrine and neuronal characteristics. They secrete neuropeptides and other hormones and often display a dendritic-like process. These can be identified using immunohistochemistry with neuroendocrine markers like chromogranin and synaptophysin.[7 10]

1.2 Prostate Organogenesis

Prostate development initiates in the later stages of embryogenesis and completes its maturation during puberty. The development and homeostasis of this gland are regulated by androgens, which are male hormones synthesised in the testicles. The production of these is strongly controlled by the hypothalamic–pituitary–gonadal axis. Leydig cells of the testes respond to the stimulation from follicle stimulating hormone and luteinizing hormone (LH) released by the anterior pituitary. LH release is controlled by pulsatile secretion of LH-releasing hormone (LHRH), also known as gonadotropin-releasing hormone from the hypothalamus. Leydig cells express the LH receptor, and LH acts on these receptors to stimulate testosterone production and promote Leydig cell growth and proliferation. Additionally, a negative feedback loop is created by inhibitory signals at the level of testosterone. Testosterone inhibits LHRH release by the hypothalamus and reduces the pituitary's sensitivity to LHRH.[12] Despite 95% of testosterone being produced primarily by the Leydig cells, there is another source. The adrenal cortex, which is part of adrenal glands, can also produce a small amount of testosterone.[13] The androgens effects occur when the testosterone is converted in dihydrotestosterone (DHT) by the 5α-reductase present in prostate cells and by this DHT binding to the androgen receptor (AR), a hormone-

transcription factor presents in both the prostate epithelium and stroma. Once they are bound, androgens activate the AR by causing its translocation from the cytoplasm to the nucleus, which, in turn, will activate genes necessary for homeostasis, angiogenesis, differentiation, proliferation, and apoptosis. Through the development, other signalling pathways are coordinated with the hormonal activity to regulate transcriptional programs that orchestrate the formation of a fully functional organ.

The first phase of the prostate begins with an endodermal urogenital sinus (UGS), an ambisexual embryonic structure that has the potential to develop into the distinct sexual organs in both female and male individuals. This tissue is walled by urogenital sinus mesenchyme (UGM), an embryonic connective tissue. It is known that in both sexes this tissue has AR, enabling it to undergo masculine, leading to the sexual differentiation. Throughout gestation, this AR activity, in response to foetal testicular androgens, initiates prostate morphogenesis. The endodermal-derived UGS epithelia (UGE) cells begin to grow into the surrounding mesenchyme, forming buds. These buds begin to elongate, leading to the formation of ducts, where the luminal and basal cells differentiate. Alongside, the mesenchyme differentiates into various cell types, including smooth muscle cells and interfascicular fibroblasts. This emphasises the significance of the interactions between the UGE and UGM. In fact, some experiments have provided valuable insights that androgens exert their effects on epithelial development primarily through AR present in the mesenchyme, mediating a paracrine action. The specific molecules produced by UGM that trigger prostatic epithelial differentiation remain unknown. However, it is believed that the induction of prostatic epithelial identity occurs as an early event preceding the formation of prostatic buds. During this developmental process, the homeobox gene Nkx3.1 becomes apparent in the UGE of male mouse foetuses approximately 48 hours before the emergence of prostatic buds. Nkx3.1 expression is regulated by androgens and is observed exclusively in the male UGE. Therefore, Nkx3.1 serves as the earliest known marker of prostatic development, indicating the acquisition of prostatic epithelial identity. The interaction between UGM and UGE is dynamic, and UGE could influence the differentiation of UGM. It can induce smooth muscle differentiation of UGM and determine its spatial pattern.[14] [15]

Throughout a man's lifetime, the interaction between the stroma and epithelium persists. Androgenic and estrogenic hormones play a key role in stimulating stromal activity, which promotes the growth and proliferation of epithelium and its secretory activity.[16]

In the postnatal era, alongside with luminal and basal unipotent progenitors, the prostate contains basal multipotent stem cells capable of differentiating into all the three types of

epithelial cells. These three cells express different markers. Additionally, there are present bipotent basal cells that can differentiate into basal cells, and intermediate cells that co-express markers associated with basal and luminal. These cells are believed to represent either multipotent prostate stem cells or intermediate cells that bridge the gap between basal stem cells and luminal progenitors, being mentioned as luminal committed basal cells (Figure 2).[17] A study have shown that basal and luminal cells can generate a complete multilayer prostate, bringing the potential of the luminal progenitor be a multipotent cell.[18]

The mature prostate, achieved in the end of puberty, is characterised by low activity, resulting in slow turnover of epithelial cells.[7] It can undergo cycles of regression and regeneration following androgen variations, implying that the prostate epithelium contains cell with a stem function.[19] The stem cells are present in both stromal and epithelial compartments and are crucial for differentiation and maintenance.[20] Lineage-tracing studies in mice have revealed that unipotent basal and luminal progenitors are primarily responsible for maintaining of their respective cell compartments during adult tissue homeostasis (Figure 2).[17] The epithelial growth, death and differentiation via stroma is mediated by "andromedins", such as fibroblast growth factor, insulin-like growth factor and epidermal growth factor. Meanwhile, ARs in luminal epithelial cells maintain cell survival whereas AR in basal/intermediate epithelial cells suppress proliferation. [21]

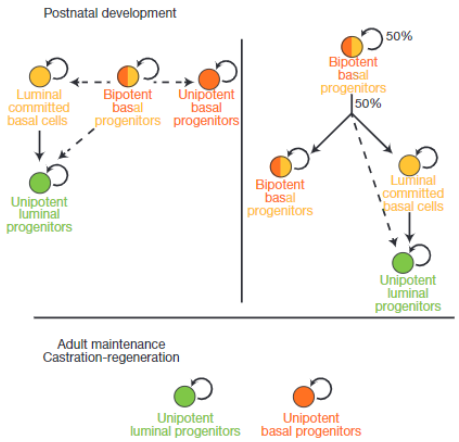

Figure 2 - The postnatal and adult maintenance of the epithelial cells in prostate tissues. [17]

Using lineage tracing and in vitro oncogenic transformation assays, luminal and basal cells have both been shown to serve as a cell of origin for PCa after an oncogenic stimulation.[22]

1.3 Pathophysiology

The transformation of the prostate into cancer follows a multistep process, starting as prostatic intraepithelial neoplasia (PIN), then progressing to localised PCa and finally to advanced prostate adenocarcinoma with local invasion, culminating in metastatic PCa (Figure 3).[23] Throughout this process, cells gradually transition from a benign to a malignant phenotype. The majority of PCa have epithelial origin, being adenocarcinomas.[24] PIN has been identified as the most likely progenitor of most prostatic adenocarcinomas. It is a premalignant condition characterised by neoplastic growth of epithelial cells, a decrease of basal cells, with an increase of luminal cells. High-grade PIN is considered a precursor to PCa, once they display an elevation of cellular proliferation markers.[25]

PCa can originate from distinct cell types.[26] Different specific genetic abnormalities have been found in human PCa including PTEN and/or p53 deletion, loss of Nkx3-1 and genetic translocations. [27] It is postulated that cancer can result from two prevailing models, which contribute to the intra-tumoral heterogeneity observed in PCa. The clonal evolution model suggests that tumours arise from a single cell of origin following a series of oncogenic changes. Inversely, the cancer stem cell model proposes that tumour cells originate from the differentiation of a rare cancer stem cells (CSCs) or from the dedifferentiation of existing cells into CSCs, promoting tumour growth and progression.[5] [28] The human PCa has a luminal phenotype, that suggests the cell of origin may be a luminal cell or a basal progenitor capable of rapidly differentiation into luminal progeny following oncogenic transformation. Tumours often display an expansion of basal cells and intermediate cells co-expressing basal and luminal markers.[26]

CSCs possess distinctive attributes such as self-renewal, pluripotency, plasticity, and the capability to restore complete tumour heterogeneity. This understanding has opened novel opportunities in therapeutics and diagnostics.[28] CSCs play a pivotal role in resistance and metastasis existing within tumours either as a subset of cells or dedifferentiating. [29] Epithelial cancer cells undergo epithelial-mesenchymal transition (EMT) to facilitate local invasion and metastasis. Neoplastic transformation via either pathway results in the emergence of genetically and phenotypically diverse cell types within the same tumour. This morphological heterogeneity accounts for the presence of multiple tumour foci within the prostate of an individual patient.[19 5]

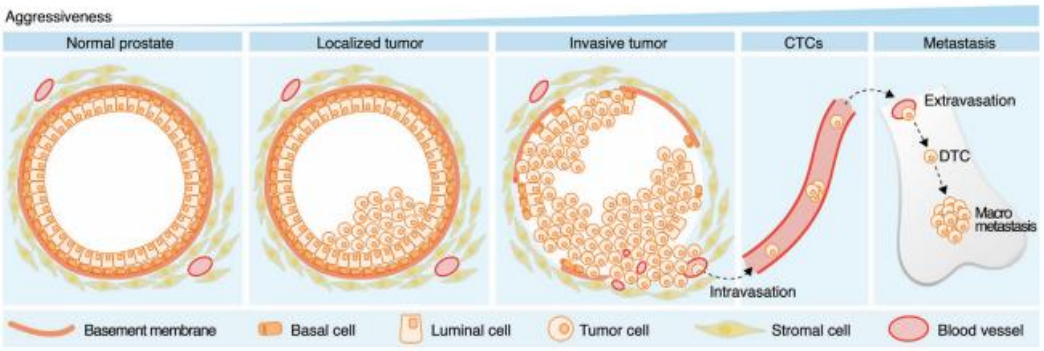

Figure 3 - Phases of PCa progression. Initially, the normal prostate transforms into a localised tumour that advances and consequently invades surrounding tissues. The tumour cells intravasation to the bloodstream, as circulating tumour cells (CTCs), to distant sites, where they can remain as disseminated tumour cells. Eventually, these cells reactivate and proliferate, leading to the formation of metastasis.[30]

1.3.1 Metastatic progression

Adding to tumorigenesis, reversible phenotypic plasticity plays a crucial role in metastasis. During the EMT, cancer cells undergo a transformation from an epithelial to a mesenchymal phenotype, which is associated with increased invasion, motility, and stem cell-like properties. However, the ability of cancer cells to undergo the reverse process known as mesenchymal-epithelial transition (MET) is also vital for successful metastasis to a secondary site. Cancer cells that can revert to the epithelial phenotype through MET, retaining plasticity, are more likely to form metastatic lesions. On the other hand, cancer cells that lose plasticity and are unable to transition back to an epithelial state are less likely to establish metastases. Studies of circulating tumour cells (CTCs) from patients with metastatic breast cancer and castration resistant prostate cancer (CRPC) also reveal that CTCs express both epithelial and mesenchymal markers, underlining the link of phenotypic plasticity of CTCs with their metastatic potential. [31]

Lymph nodes close to primary tumours often serve as the initial metastatic sites. Lymphatic vessels have a distinct structure, with no tight interendothelial junctions. The vessels become permeable under increased tissue pressure, enabling tumour cells and signalling molecules entry. From lymph nodes, they travel to regional and distant lymph nodes, eventually reaching the bloodstream, where they have the potential to metastasize to distant organs. Many tumours secrete growth factors that act in a paracrine manner, stimulating the lymphangiogenesis, promoting the lymphatic vessel growth and metastasis lymph node metastasis.[32 33]

PCa bone metastases manifest as osteoblastic lesions with mixed osteolytic features, leading to severe pain, hypercalcemia, and frequent fractures.[34 35] After PCa cells metastasize to the bone marrow, interactions between the cancer cells and the bone microenvironment

trigger a continuous cycle of bone formation and destruction, fostering cancer cell survival and tumour growth.[36] Inflammatory pathways and physical forces stimulate osteocytes to secrete tumour-promoting factors, like matrix metalloproteinases, supporting the metastatic growth. This growth of PCa cells in the bone involves a dynamic process of bone remodelling, driven by interactions between cancer cells, osteoblasts, and osteoclasts.[37]

Primary tumours can prepare premetastatic sites and exploit immune cell types to aid metastasis. Macrophages, platelets, and mesenchymal stem cells (MSCs) facilitate the EMT at the primary tumour site. This transition allows tumour cells to detach from neighbouring epithelial cell-cell connections and acquire a mobile and invasive phenotype. Tumour growth factor-beta (TGF-β), secreted by the tumour stroma, acts as a crucial mediator in this process through paracrine signalling with tumour cells. Tumour-associated macrophages (TAMs), cancer-associated fibroblasts (CAFs), and myeloid progenitor cells are observed to accumulate at the tumour's invasive edge. This gathering contributes to immunosuppression by interfering with dendritic cell differentiation. During the intravasation phase, macrophages located in perivascular areas aid tumour cells in overcoming vessel barriers. In the bloodstream, platelets and coagulation system components act as shield for tumour cells, preventing immune recognition while circulating. Platelets play a guiding role, directing tumour cells to exit blood vessels at secondary sites by interactions with areas of vascular retraction. at secondary sites, fibroblasts enhance fibronectin expression. This creates a docking site for hematopoietic progenitor cells and aids the infiltration of tumour cells. Immunosuppressive cell types like myeloid-derived suppressor cells (MDSCs) and natural killer cells accumulate in premetastatic niches, creating a supportive environment for tumour establishment and spread.[38]

Communication between the primary and the secondary sites occurs through exosomes, released by primary tumour cells, immune cells, and stromal cells. These exosomes carry factors influencing organ tropism, immune evasion, MET, and can predict metastasis and patient outcomes. [38]

1.3.2 Tumour microenvironment

The tumour microenvironment (TME) is a complex and highly dynamic network that significantly influences the cancer progression and treatment response. Besides the epithelial cells, the TME includes blood vessels, lymph vessels, CAFs, immune cells, nerves, and extracellular matrix (ECM), all of which collectively constitute the tumour stroma. As mentioned previously, in the prostate, the stroma and the epithelium are closely linked, and they are dependent on each other for development and function of the normal prostate. This communication remains crucial in PCa, where the epithelial changes affect the stroma and vice

versa. The tumour stroma can either suppress or promote cancer growth. However, the presence of cancer cells triggers significant changes in the stromal environment, to create a supportive milieu for tumour growth.[39] These significant changes on gene and protein expression of stroma during PCa translate into the tumour aggressiveness and patient outcome. Several significant changes in the tumour stroma can be observed: [40 21 39]

1. Transition from smooth muscle cells to CAFs

The gradual replacement of smooth muscle cells by fibroblast, in this case CAFs, results in an altered expression of markers, desmin and smooth muscle actin are replaced by markers such as vimentin. The origin of CAFs remains unclear, and they could be produced by activation of pre-existing fibroblasts and pericytes, recruitment from the bone narrow, or transdifferentiation of epithelial or endothelial cells. Consequently, different subpopulations of CAFs may have diverse origins expressing various markers. CAFs can affect cancer cells and other tumour compartments by secreting growth factors, EMC components, and proteases. CAFs are thought to interact with the tumour cells and form an uncontrolled "reactive stroma" that stimulates cancer cell proliferation and aggressiveness, therefore affecting response to treatment. They are not the same in all individuals and the CAFs are dependent on the tumour epithelial cells. [21 39]

2. Enhance vascular formation

An increased formation of new blood and lymph vessels is observed due to the altered release of regulators that stimulate vascular growth. These regulators of vascular growth are originated from the tumour epithelium and the tumour stroma. The formation of blood vessels is crucial to support cancerous cell viability and growth. [21 39]

3. Influx of inflammatory cells

Inflammatory cells, such as macrophages, lymphocytes and mast cells infiltrate the normal tissue adjacent to the tumour and into the tumour stroma. These cells secrete cytokines that have stimulatory or inhibitory effects on adjacent CAFs, blood vessels and tumour epithelial cells. The intensity of these inflammatory processes impacts the tumour aggressiveness. [21 39]

4. Hypoxia

Hypoxia is a prominent pathological feature within solid tumours. Tumour hypoxia has been recognized as a significant contributor to treatment resistance and the subsequent progression of lethal diseases, including metastatic PCa. This is attributed to the fact that hypoxia triggers a diverse group of genes and corresponding pathways that facilitate stress

adaptation and survival. Consequently, cancer cells exposed to hypoxia are more inclined to withstand treatments and proliferate in contrast to their normoxic counterparts. In response to hypoxia, hypoxia-inducible factor is activated and triggered, primarily serving as the master transcription factor responsible for upregulating these genes.[41]

5. Increase of TAMs

TAMs play crucial roles in tumorigenesis. They can originate either from tissue-resident macrophages or from peripheral reservoirs like the bone marrow and spleen and tend to accumulate in hypoxic regions within growing tumours. Their recruitment is facilitated by the upregulation of macrophage chemoattractant, including endothelin-2 and VEGF, which correlates with angiogenesis and the acquisition of an invasive phenotype. This suggests that the initial hypoxic response in growing tumours may trigger a switch in macrophage polarisation, leading to their supportive role in tumour progression. [21 39]

6. Alterations in ECM composition

The ECM composition changes, with components produced by CAFs and tumour-infiltrating macrophages. That contains collagenous fibres and multiple non-collagenous proteins such as fibronectins, bone sialoproteins, osteocalcins, cadherins, osteonectins, and vitronectins. The metastatic progression is dependent on a breach of the barrier formed by this matrix. The ECM not only provides biochemical signals but also biophysical cues, such as matrix rigidity, that significantly influence cellular behaviour. This can affect various aspects of cancer cells, including proliferation, stem cell properties, and metastatic growth. As PCa becomes more aggressive, collagen fibres tend to be more oriented, resulting in a stiffer matrix. [21 39 42]

The composition of the TME can vary greatly between patients and even within the same patient, leading to significant disease diversity. This diversity presents challenges in tailoring the most effective treatment plans for individual patients.[42]

1.4 Detection

Recurrent screening programs for PCa diagnosis have been implemented with the objective to detect the disease at an early stage, leading to improved survival rates and quality of life. These programs are designed to perform regular screenings on individuals, particularly those with higher risk. An early detection allows the individuals have better treatment options and outcomes.[43] Men who are over 50 years, those over 45 years old with a family history of PCa or African-American, and BRCA/2 carriers over 40 can be selected to early detection.[44]

The PCa can be suspected based on the results of a digital rectal examination (DRE) and PSA levels. However, a definitive diagnosis requires histopathological confirmation of adenocarcinoma in prostate biopsy samples.[44] The DRE is conducted by a healthcare professional that inserts a lubricated, gloved finger into the rectum to evaluate the size, shape, and texture of the prostate. If the examiner detects any abnormalities such as induration, nodularity, significant asymmetry or loss of anatomic landmarks, the DRE is considered positive or suspicious. This test is subjective, so the combination with the PSA screening helps to a significantly detection of PCa and to decrease unnecessary biopsies and overdiagnosis.[45]

Typically, the evaluation of blood samples for PSA is the spine of PCa diagnosis. PSA is a glycoprotein that is predominantly produced and secreted by prostatic epithelial cells. Its primary functions include liquefying serum, promoting sperm motility and dissolving cervical mucus.[46] Usually, only a small amount of PSA is released into the bloodstream. However, conditions such as BPH, prostatitis, PCa can lead to increased levels of PSA in the circulation. PSA is present in different forms in the serum. The majority of PSA exists as complexes with alpha-1-antichymotrypsin, which accounts for 70-90% of PSA in the bloodstream. The remaining portion, approximately 10-30%, is unbound and referred to as free PSA. This is the portion of PSA that is not bound to the serum protease inhibitors and cannot be detected by immunoassays. On the other hand, PSA bound to alpha-2-macroglobulin can only be measured if the complex is cleaved, allowing the PSA epitopes to become accessible for detection. PSA is widely recognized as a "biomarker" or "tumour marker" due to its ability to provide insights into the biological state of PCa.[47] Specific antibodies have been developed to accurately measure both the free form of PSA and total PSA, which includes both free and complexed PSA. Free PSA is now identified to exist at least in three distinct forms of inactive PSA. Among these forms, one recognized as the proenzyme or precursor forms of PSA and is linked to cancer. Another form, called BPSA, is a degraded version of PSA that is more predominantly associated with BPH. The third PSA form may include several minor variants, but predominantly consists of intact PSA that is structurally like active PSA, although it has lost its enzymatic activity due to certain structural or conformational changes.[48] Initially, a PSA cut-off of ≤4ng/ml was suggested as a normal level for men aged 50–70 years. However, further analysis of men with a PSA level of ≤4.0ng/ml in the Prostate Cancer Prevention Trial revealed that 15% of them had clinically significant PCa.[49] For PSA levels between 4.0 and 10.0ng/mL, the positive predictive value is about 25%. However, nearly 75% of cancers detected within the "Gray zone" of PSA values between 4.0 and 10.0ng/mL are organ confined and potentially curable. [50] In addition to the traditional PSA levels, other forms of PSA measurement can be utilised, including free/total PSA ratio, PSA density and PSA velocity. A higher ratio of free to

total PSA in the bloodstream is associated with a reduced risk of PCa. PSA density involves dividing the serum PSA level by the estimated prostate volume, typically determined through transrectal ultrasound (TRUS). This helps account for variations in gland size when interpreting PSA values. PSA velocity, on the other hand, assesses the longitudinal change in PSA values over a specific period. It can be used to predict the presence and aggressiveness of PCa before initiating definitive therapy.[51]

The use of imaging in PCa diagnostics is continuously advancing, typically used in conjunction with the previous methods, providing the ability to detect clinically significant PCa and reduce unnecessary biopsies. TRUS is commonly employed in diagnosing PCa particularly for targeting specific anatomical regions or visible lesions.[52] But is not considered a reliable method for detecting PCa, due to TRUS cannot reliably detect lesions that prove positive on biopsy, which is why it is always used in conjunction with biopsy. Although PCa can occasionally appear as a hypoechoic lesion on grayscale TRUS, the absence of such lesions does not exclude the possibility of cancer.[53] Biopsies conducted on these lesions have shown limited diagnostic success. The limitations of this method result from two primary factors. First, there are similar backscatter signals observed from PCa and normal prostate parenchyma, making it challenging to differentiate between the two. Second, the heterogeneity of the transition zone further complicates the accurate identification.[54] However, there are emerging sonographic techniques such as micro-Doppler, sonoelastography, and contrast-enhance ultrasound that have exhibited promising results. These modalities can be employed individually or combined in a technique known as "multiparametric ultrasound".[44]

Multiparametric magnetic resonance imaging scanning (mpMRI) is currently the most accurate imaging technique for detecting potential PCa, determining the stage of the disease, and for monitoring patients under active surveillance (AS). This method utilises high-resolution images obtained through three different contrasts, or MRI sequences, known as "multiparametric".[55] In addition to providing detailed anatomical information, mpMRI also reveals tissue characteristics such as prostate volume, cellularity, and vascularity, enhancing its diagnostic capabilities.[56] A mpMRI should be considered before a prostate biopsy. In a case that needs a biopsy these two imaging techniques could be used to guide.

Recently a new diagnosis method has been used that combines a biomarker and imaging technique, the prostate-specific membrane antigen (PSMA) positron emission tomography/computed tomography (PET/CT). PSMA is a transmembrane protein that is expressed in the epithelium of the prostate but not specific to this organ, being produced in other glands.[57] In PCa this type 3 protein is overexpressed, and such presence increases with

the increase of PCa and tumour grade.[58] PSMA ligands used for PET/CT imaging bind to the surface receptor and internalise within cells. As the other imaging methods PSMA PET can be used to detect the need for and guide, a targeted biopsy in patients presenting with clinically suspected PCa. Moreover, PSMA PET has the potential to enhance the precision of tumour segmentation before radiation therapy or other localised treatments. This method enables a non-invasive characterization of ambiguous findings and offers valuable prognostic information.[59 60]

As previously mentioned, a biopsy is usually performed to confirm the presence of cancer, the last step of a process, this method collects a small tissue sample from the gland to be observed and classified. There are two primary methods used to obtain prostate tissue for detecting PCa: Transperineal (TP) biopsy and Transrectal (TR) biopsy. These approaches differ in various aspects, including the puncture site, puncture route, and the type of TRUS transducer used. Currently, there is a lack of standardisation between TP and TR biopsies regarding the rates of PCa detection. In TR biopsy, the needle is guided through the anterior rectal wall using an end-fire transducer. On the other hand, in TP biopsy, the needle is directed through the perineal skin under the guidance of a bi-planar transducer. [61]

The most used system for PCa stratification is the Gleason system (Figure 4). This histologic grading system is only based on the pattern of organisation of carcinoma cells and provides a correlation between histologic grade and biological malignancy. Due to PCa being highly heterogeneous it is considered and classified in a range from 1 to 5 the two most prevalent tumour patterns. A well-differentiated process of PCa is identified by the presence of proliferating microacinar structures that are lined by prostatic luminal cells, lacking an accompanying basal cell layer. The primary and secondary grades, with 1 being the most differentiated and the 5 the least differentiated, are combined to determine the Gleason score that consequently has a possible range from 2 to 10. There are some exceptions, sometimes only the primary pattern is in the tissue, so the grade must be multiplied by two, other exceptions is when the second grade is less than 3% of the total tumour, in this case this is ignored, and the primary grade is doubled. A Gleason score below 6 indicates a less aggressive malignancy with a favourable prognosis, while a Gleason score above 8 is associated with a more aggressive behaviour and an elevated risk of undetected systemic disease. [62] However,

this system has limitations since it will depend on the size of the tumour sample and the size of the tumour itself. [63 64 65]

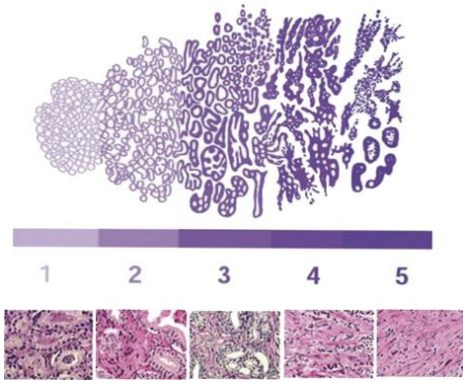

Figure 4 - Diagram illustrating the Gleason grading system. The numbers are referent to Gleason grades, above are the respective Gleason drawings of each grade and below the corresponding stained micrographs for each grade. [62]

There are other classifications systems that are commonly used for PCa in clinical practice such as International Society of Urological Pathology (ISUP) and TNM. The first one arises from the necessity of specifying morphologic features in certain patterns from Gleason score.[66] This system grades the cancer between 1 and 5 for the following Gleason scores ≤6, 3 + 4 = 7, 4 + 3 = 7, 8 and 9–10, respectively.[67] TNM is the most common system being used in diverse types of cancer. The T corresponds to the size and extent of the primary tumour and goes from 1 to 4. The N stands for the number of nearby lymph nodes with cancer and M to the metastasis, which means that the cancer cells have spread from the main tumour to other parts.[68]

1.5 Therapy

A pathology report should contain histopathological (sub)type (conventional acinar adenocarcinoma, neuroendocrine cell carcinoma or ductal carcinoma) and grade. It should also provide details about pathological staging, clinical stage (Table 1), surgical margins, and other relevant factors. These parameters considered in conjunction with several factors like PSA levels, baseline urinary function, comorbidities, and age, define the aggressiveness and stage of the cancer, where the treatment decision is based (Figure 5).[44]

Localised PCa is characterised by cancer that is limited to the prostate gland and has not spread nearby tissues or distant locations. According to the TNM staging system, localised PCa is commonly categorised as stages 1, 2 or 3 (Table 1). Treatment options for localised PCa may include prostatectomy, radiation therapy, expectant management, or a combination of those. When the cancer cells spread beyond the prostate gland to nearby tissues. We are in the presence of a locally advanced PCa. The treatment options may include hormone therapy,

radiotherapy, chemotherapy, targeted therapy, immunotherapy, or a combination of these approaches. In the following table is explicit how the different classifications systems work.[44]

Table 1 - Stratification of non-metastatic PCa based on different types of tumour classifications. Adapted from[44]

	Localised			**Locally Advanced**
	Low risk	Intermediate risk	High risk	
PSA	< 10 mg/ml	10 - 20	> 20	any
GS	< 7	7	> 7	any
ISUP	1	2/3	4/5	any
TNM	cT1-2a	cT2b	cT2c	cT3-4 or cN+

Metastatic PCa refers to cancer that has spread to distant sites in the body, such as bones. Metastasis occurs when cancer cells break from the primary tumour and travel through the bloodstream or lymphatic system to establish new tumours in other sites. This type of PCa requires systematic treatment approaches, including hormone therapy, chemotherapy, targeted therapy, immunotherapy, and radiopharmaceuticals. Treatment aims to control the spread of the cancer, relieve symptoms, and extend survival.[44]

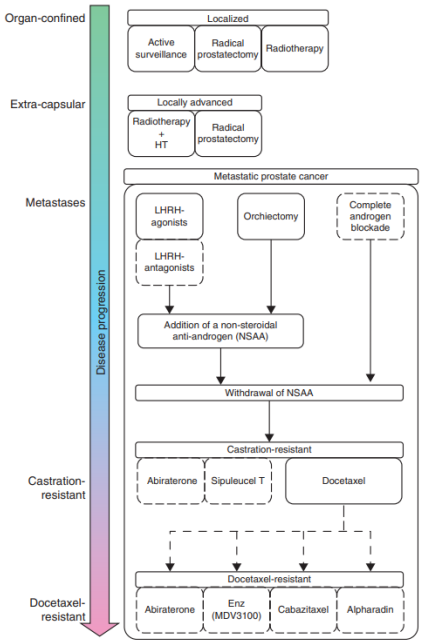

Figure 5 - Overview of the development of PCa therapy along the disease progression. [69]

1.5.1 Deferred Treatment

There are two conservative approaches aimed at reducing the over-treatment in PCa management. One approach is AS, which seeks to avoid unnecessary treatment and its associated side effects. AS is recommended for men with clinically localised PCa, a life expectancy of 10 years or more, and who do not require immediate treatment. Its objective

is to achieve the appropriate timing for curative treatment. AS is gradually being recognized as a strategy to prevent unnecessary interventions by allowing a shift to active treatment when considered necessary.[70] It involves regular monitoring through PSA testing, physical examinations, prostate biopsies, or a combination of these methods to detect any progression and provide curative treatment for those with significant disease. On the other hand, watchful waiting is a conservative management for patients who are initially considered unsuitable for curative treatment. These patients are closely observed for the development of local or systemic disease progression and the emergence of disease related symptoms. When symptoms arise or become imminent, palliative treatment is initiated to alleviate those symptoms and maintain the patient's quality of life, that has not curative intention. This approach focuses on managing symptoms with palliative intent and is intended for patients with a limited life expectancy.[71]

1.5.2 Prostatectomy

A radical prostatectomy is a surgical procedure that involves the removal of the prostate gland, along with the surrounding tissues. There are two main approaches: the traditional radical approach and minimally invasive techniques such as laparoscopic or robotic assisted surgery. These minimally invasive techniques have been applied to prostatectomy to further decrease complications and improve the healing process.[72] Compared to open surgery, laparoscopy and robot-assisted prostatectomy typically result in less blood loss, shorter hospital stays, and quicker return to work. They have demonstrated favourable oncologic outcomes to those achieve with open surgery. However, the functional outcomes related to urinary continence and erectile potency could be better. [73]

1.5.3 Radiation Therapy

Radiation therapy is a highly effective treatment option for the majority of men diagnosed with localised PCa. Different forms of radiation therapy have demonstrated their effectiveness in treating and achieving cure for this disease.[74] The standard treatment approach is the external beam radiotherapy using conventionally fractionated radiotherapy at a dose of 76-80 GY and a schedule that lasts over 7-8 days.[75] In nowadays, Intensity-modulated radiation therapy (IMRT) and volumetric arc radiation therapy (VMAT) with image-guided radiation therapy are widely recognised as the standard treatment approach for EBRT. VMAT offers the advantage of shorter treatment times than IMRT, usually two to three minutes in total. Both techniques allow for a more complex distribution of the dose to be delivered and provide concave isodose curves, which are particularly beneficial for sparing the rectum.[44] Recent

advancements in radiation therapy have enabled more precise targeting of the prostate gland while reducing the impact on nearby organs and tissues, these include brachytherapy (BT), intensity-modulated radiotherapy and stereotactic body radiotherapy.[74] BT, which involves either low-dose-rate (LDR) permanent seed implantation or high-dose-rate (HDR) temporary source implantation, is considered a suitable treatment choice for specified patients with PCa, regardless of their risk group. HDR-BT offers certain benefits compared with LDR-BT. These include the ability to use the same radiation source for treating other types of cancers, reduced operator dependence, and normally less acute irritative symptoms.[76] It can be used in combination with other therapies. Salvage radiation therapy (SRT) is an approach employed when local recurrence of PCa is identified, usually indicated by rising levels of PSA with or without evidence of clinical recurrence. SRT aims to target and treat the recurrent disease. On the other hand, adjuvant radiation therapy (ART) refers to radiation treatment delivered after radical prostatectomy to eradicate any presumed microscopic disease before PSA failure occurs. ART is primarily proposed to individuals considered at high risk of recurrence following surgery, such as those with locally advanced or margin-positive disease. The objective is to eliminate any remaining subclinical disease, potentially delaying or preventing further biochemical and clinical recurrence.[77] The association with androgen deprivation therapy (ADT) is detailed in EAU Guidelines for PCa and is used particularly in cases with significant disease. Randomised clinical trials have confirmed the benefits of short-term (6 months) ADT for intermediate-risk disease and long-term (24 months) ADT for high-risk disease.[78 79]

The Prostate Testing for Cancer and Treatment, a trial randomised study of 1643 men in the United Kingdom who underwent screening for localised PCa, were assigned in three groups: active monitoring, surgery, and radiation therapy. The trial found that men who opted for AS maintained a better quality of life. However, half of the men in AS eventually end up receiving treatment. Both surgery and radiation compared with AS demonstrated a reduction in the risk of clinical progression and metastatic disease. These findings suggest that there may be potential differences in mortality outcomes with longer-term follow-up.[80]

In a more recent trial that included new techniques such as brachytherapy, it was observed that radical prostatectomy was associated with sexual dysfunction and urinary incontinence. On the other hand, radiotherapy was linked to short-term urinary obstruction, irritation and bowel symptoms compared to men undergoing AS.[81]

1.5.4 Focal Therapy

Focal therapy has emerged as a new approach for the treatment of PCa, aiming to reduce treatment related morbidity while targeting specific tumours. Various techniques, including

cryotherapy, high-intensity-focused ultrasound (HIFU), laser ablation, brachytherapy, and other energy-based methods, have been explored in this context. HIFU involves the use of focused high-intensity ultrasound to raise the temperature above 60°C, resulting in tissue ablation. This process involves two mechanisms: coagulative necrosis caused by extreme heat and internal cavitation resulting from the interaction between water and ultrasound waves. On the other hand, cryotherapy utilises extreme cold temperatures to induce tissue ablation. It employs cryo-needles placed in the target area through the perineum. By maintaining a specific distance between the needles, a uniform ice ball is formed, ensuring complete coverage of the targeted region. Cryotherapy achieves tissue ablation through mechanisms such as osmotic injury, cytolysis, apoptosis, and vascular damage. Focal therapy appears to be safe with favourable preservation of genito-urinary function. However, existing cohort studies primarily include men with less-aggressive cancer, and the reported success rates vary, with residual tumour found in 5.1% to 45.9% of cases (0%-13.4% with significant disease).[82]

1.5.5 Hormonal Therapy

ADT is the first-line treatment for men diagnosed with metastatic PCa. ADT results by reducing the secretion of testicular androgens through various methods, which can be surgical or chemical. As previously said the androgens have a major role in the development of the prostate gland and in the prostate carcinogenesis. Therefore, the AR control is a key target for systematic therapy in PCa. In normal prostate tissue, the AR maintains a balance between cell differentiation and proliferation. However, as PCa progresses from early to advanced stages, this balance shifts in favour of increased cell proliferation. This shift is accompanied by the downregulation of genes that promote differentiation and upregulation of genes that drive proliferation. ADT induces a systemic decrease in circulating androgens creating an androgen-depleted environment that prevents activation of the AR, that leads to the prostate involution and apoptosis of epithelial cells. Surgical castration, which involves bilateral orchiectomy, eliminates the major source of androgens, contributing to the desired androgen-depleted state for PCa treatment.[44] [83] ADT offers several benefits, that include tumour regression, extended overall survival, improved cancer-specific survival, and relief of urinary symptoms, improving the quality of life for patients.[84]

Chemical castration has the advantage of reversibility and avoiding the body dysmorphia associated with surgical castration, consequently, making it the primary approach of ADT. ADT can be accomplished using either LHRH agonists or antagonists. LHRH agonists initially lead to an increase in LH and testosterone levels, but due to the negative feedback loop, they eventually lower testosterone levels to "castrate" levels (< 50ng/dL) within about four weeks

of treatment. However, in patients with metastatic PCa, concerns about the initial surge in testosterone and its consequences need to be addressed. To mitigate this, LHRH agonists are often preceded by direct AR antagonists, which block the downstream effects of androgens. On the other hand, LHRH antagonists inhibit LH production and subsequently testosterone synthesis, achieving castrate testosterone levels within three days of treatment. While LHRH agonists and antagonists can inhibit androgen production by the testicles, other cells in the body, such as the adrenal glands and PCa cells themselves, can still generate testosterone. Certain drugs target the androgen axis, preventing the synthesis of androgens by these cells. This includes inhibitors of cholesterol synthesis steps like cytochrome P450 (CYP-17) inhibitors and nonsteroidal azoles. Furthermore, competitive AR antagonists effectively block testosterone from binding to its target receptor and activating it. Since androgens play essential roles in various physiological processes, ADT is associated with a notable side effect profile. Common adverse effects encompass "hot flashes," the development of gynecomastia, sexual dysfunction, bone loss and associated complications, anaemia, cognitive effects, and metabolic changes.[85]

Castration Resistant Prostate Cancer

Despite ADT being commonly used to treat patients with metastases, it is not curative. After a variable period of treatment, typically ranging from 2 to 3 years, most of all patients with metastatic PCa develop resistance to this therapy. CRPC is characterised by cancer progression with low levels of testosterone, consecutive rises in PSA and radiological progression.[86]

Molecular biology of development of CRCP

These cases of PCa there are a more aggressive and metastatic phenotype.[87] Several mechanisms of resistant promotes the progression from hormone-dependent to CRPC. Mechanisms encompass AR amplification, hypersensitivity, mutations, alterations in coactivators/corepressors, androgen-independent AR activation, intratumoral and alternative androgen production, as well as AR variants.

The AR overexpression due to a gene amplification sensitises PCa cells to low androgen levels, which enables CPRC to be hypersensitive to these levels and promote the progression of disease.[88] Furthermore, identified mutations enhance AR activity by recruit coactivators, changing the ligand affinity or specificity, or causing antagonist-to-agonist switch. This induces the response of two non-androgenic steroids, such as progesterone, hydrocortisone, oestradiol, and certain AR antagonists. Mutation of the coregulators of AR, which can be co-

activators or co-repressors, is another mechanism. There are several studies that have identified and demonstrated important activity in CRPC, such as FKBP51 and SRC class, two coactivators.[89] [90] In general, the coactivators are upregulated and the corepressors in reduced levels.

As previously said, the testes are not the only producer of androgens, the adrenal glands and PCa cells can do it. So, the CRPC can resist by synthesising their own androgens, through de novo synthesis from cholesterol or by metabolising weak androgens via a "backdoor" pathway, ultimately converting them to highly active DHT. [91]

Aberrant activation can occur, this mechanism consists in a ligand-independent AR activation. Various factors like growth factors, cytokines, and kinase pathways can increase AR signalling, promoting progression of CRCP. For example, NF-κB/p52, activates the AR, enhancing nuclear translocation by interacting with its N-terminal domain. p52 facilitates the recruitment of coactivators such as p300 to the promoters of AR-dependent genes, resulting in improved transactivation of AR-responsive genes in androgen-deprived conditions.[92]

The AR splice variants are another mechanism, these variants are constitutively active, maintaining the transcriptional core segments while modifying ligand-binding domain, that promotes the insensitive to AR agonists or antagonists.[93]

The capacity of PCa cells to undergo dedifferentiate into CSCs plays a crucial role in developing resistance to AR-antagonists. This process allows reprogrammed cells, when exposed to androgens, to reactivate the AR signalling pathway. This phenomenon indicates the dynamic nature of the cellular plasticity, showcasing the cells' ability to respond to the external cues and adapt to changing conditions.[5]

Neuroendocrine PCa is considered as a mechanism of resistance against standard hormonal treatment. This highly aggressive PCa subtype can arise *de novo* or develop in patients who have previously undergo hormonal therapies for prostate adenocarcinoma.[94] NE cells exhibit a lack of PSA or AR expression, rendering them resistant to hormonal therapies targeting AR signalling. This resistance supports the NE cell survival and enrichment, potentially aiding the survival of adjacent luminal tumour in an androgen-deprived environment, contributing to the treatment resistance. The origin of small cell neuroendocrine carcinoma remains uncertain, whether arising from transdifferentiation of luminal cells or NE cells clonal expansion. NE phenotype induction can result from various stimuli and signalling pathways, suggesting it might be a default state under stress. The concept of transdifferentiation implies that NE tumour cells might arise from luminal cells following

hormonal therapy. [95] As a result, its marker, chromogranin A, is associated with the development of CRPC and shortened time to disease recurrence.[27]

As a result, there is a need for additional treatments to address CRPC and its progression. One viable therapy is the use of CYP17 inhibitors. As previously mentioned, these inhibitors can block the formation of androgens in tissues other than gonads. This enzyme is responsible for catalysing the conversion of the precursors of testosterone and DHT in both adrenal and intratumoral synthesis. [86]

1.5.6 Chemotherapy

The chemotherapy is typically used in cases of metastatic PCa.[44] Among the frequently used drugs are docetaxel and cabazitaxel, both belonging to the taxanes class. Docetaxel is a highly cytotoxic semisynthetic taxane that inhibits microtubular depolymerization, causing cell arrest in the G(2)M phase of the cell cycle, leading to a cascade of events that end in an apoptotic cell death. This can be used with other drugs, such as prednisolone in metastatic CRPC and ADT for hormone-sensitive metastatic PCa.[96] Cabazitaxel is a microtubule inhibitor.[97] In vitro studies have demonstrated that all taxanes exhibit a certain level of cross-resistance, but cabazitaxel showed the lowest rate. This crucial characteristic justifies the use of cabazitaxel as a treatment option for CRPC previously undergone a docetaxel-containing treatment regimen.[44]

1.5.7 Immunotherapy

The tumour organisation can significantly influence immune inhibition and evasion mechanisms. Cancer vaccines aim to activate tumour-specific T cells by upregulating the initial step of antigen presentation. However, a single vaccine may not be enough to fully activate T cells for an effective anticancer response. The effectiveness of these vaccines may also be influenced by cytokine levels. Some bio-immunotherapies have shown promise overcomes immune evasion mechanism. Nowadays, the Sipuleucel-T is the only approved agent for treating disease. It is the first-line treatment option in patients with chemotherapy-naïve, asymptomatic, or minimally symptomatic advanced PCa.[98] Sipuleucel-T consists of autologous peripheral-blood mononuclear cells, including antigen-presenting cells (APCs), that have been activated ex vivo with a recombinant fusion protein (PA2024). This protein is a prostate antigen, prostatic acid phosphatase, fused to granulocyte–macrophage colony-stimulating factor, which is an immune-cell activator. [99]

The immune response against tumours involves capturing neoantigens by APCs and presenting them to cytotoxic T lymphocytes, leading to T cell priming and activation. Effector

T cells then travel to the tumour site to identify and eliminate cancer cells. However, cancer cells can evade this response through downregulating surface antigens, releasing immunosuppressive cytokines, and producing molecules like PD-L1/L2 that bind to PD-1 on T cells and dampen their activity. The tumour microenvironment can contain suppressive cells like MDSCs and regulatory T cells, further inhibiting the antitumor immune reaction. Tumour-infiltrating lymphocytes may excessively express CTLA-4, negatively affecting T cell function. Immunotherapeutic strategies are being developed to counter these evasion mechanisms and target different stages of the cancer immunity cycle. Investigational approaches for PCa include checkpoint blockade with anti-CTLA-4 and anti-PD-1 antibodies, as well as chimeric antigen receptor T cells that specifically target cancer-associated antigens to overcome immune suppression.[100]

1.6 In Vitro 3D Models for Prostate Cancer

To enhance the understanding of disease the experimental models are a crucial tool. An ideal model should be both reproducible and accurate in mimicking human disease behaviour. Biomedical research had a shift driven by three key factors: the lack of suitable animal models to replicate human conditions due to genetic variation, ethical concerns and cost associated with animal testing, and the progress in cell culture research.[101] In vitro models that can analyse cell growth interaction, homeostasis, EMT, invasion and metastasis, are becoming increasingly valuable for basic research and the advancement of novel therapeutic approaches.[102] In the PCa the resistance to the therapies, such as ADT, chemotherapy and immunotherapy, is common and an object of research.[23] There are various in vitro models to address PCa.

Traditionally, preclinical investigations of PCa have relied on 2D cultures of cell lines. In these cell-based models, the cells are cultured as a monolayer in a specific medium that supports their growth and development. They can replicate the various stages of PCa, giving valuable insights into cancer biology, drug evaluation and gene expression. The 2D models have the advantage of low cost, high reproducibility, simple methodology and easy equipment requirements. Although, they display some limitations, such as lack of vasculature, absence of native microenvironment reproduction and lack of cell interaction with the extracellular matrix and other cells.[103 104] To overcome these limitations, more advanced models have been used. 3D models have emerged as a promising approach, bridging the gap between traditional 2D cultures and live tissues. [105 106]

Compared to conventional 2D cell cultures, 3D cell cultures have gained prominence for their ability to faithfully replicate the complex *in vivo* cellular environment. This enhanced

mimicry makes them highly effective in studying cellular behaviour and a range of biological processes, such as the TME and its influence on cancer behaviour and therapy responses. These 3D cultures can be established in various formats, including spheroids, organoids, and scaffold-based cultures, offering flexibility to researchers. The utility of 3D cell cultures extends across diverse biological phenomena such as cancer progression, tissue development, and drug metabolism. Importantly, these cultures hold promise as an ethical alternative to animal testing, given their capacity to provide more accurate and relevant results.[104] [107] There are different types of 3D models.

1.6.1 Spheroids

Spheric cancer models, such as spheroids, tumorspheres, and organoids, are derived from cancer tissues or cell lines. Tumour spheroids, also known as tumoroids, are multicellular aggregates obtained from immortalised cancer cell lines using the "hanging drop" method or non-adherent cell culture plates. Tumorspheres, on the other hand, are derived from CSCs isolated from cell lines or tumour tissues and processed through the "hanging drop" method or in non-adherent cell culture plates and processed through mechanical and enzymatic dissociation. Organoids, which resemble the microanatomy of corresponding organs, are generated from circulating tumour cells or dissociated tumour biopsies and are seeded as single cells in growth. They exhibit the histological and molecular epithelial features of their original tumours, recapitulating the diversity of PCa subsets, although they may lose certain microenvironment elements over time. Overall, spherical cancer models, alone or in combination with bioengineered systems, have been applied.[108] [109]

1.6.2 Organ explants

This model involves cultivating explanted tissues, such as primary tumours or bone fragments of murine or human origin. The thickness of these tissues can vary, ranging from thin slices to larger fragments. The main advantage of these systems is that they maintain the complexity of the in vivo tissue, including its 3D architecture and various cell types. Although ex vivo tissue survival is typically limited to a few days, this can be extended using methods like specific solutions, bioreactors, or gelatine sponges. Explants have been employed to study therapeutic and examine specific disease states, such as CRPC and metastatic tumour study the therapeutic response to antiandrogen and antiangiogenic agents.[110]

1.6.3 3D Bioengineered Models

These biomimetic models are recent, developed by tissue-engineered platforms, utilise different biomaterials of natural or polymeric origin, organised into structures like meshes or sponges, to mimic the 3D microenvironment of PCa in vitro. The 3D bioengineered systems successfully replicate various aspects of tumour progression, such as initiation, growth, migration, invasion, interactions with stromal cells, and mechanisms of drug response and resistance.[110]

1.6.4 Bioreactors

A bioreactor is a sophisticated system designed to cultivate cells under precise and tightly controlled conditions, including pH, temperature, pressure, and nutrient levels. It offers the ability to regulate multiple stimuli, such as growth, mechanical, or electrical cues, to simulate physiological processes, thereby overcoming the limitations of conventional cell cultures. With the use of bioreactors, researchers can study cell and tissue functions within a well-defined and controlled 3D environment. These bioreactors have been successfully employed to create large tumour cell aggregates and model liver metastasis, providing valuable insights into complex biological processes.[111]

1.6.5 Microfluid Devices

Microfluidic devices, often referred to as 'organ-on-a-chip', are artificial and miniature models of organs designed in a 3D microfluidic cell culture device. These systems aim to replicate essential characteristics of native tissues, including the flow of fluids and the cellular microenvironment, encompassing cell-cell and cell-extracellular matrix interactions, as well as spatiotemporal chemical and physical gradients. The development of organ-on-a-chip models ensures proper support for 3D tissue culture and precise control over physical factors that impact the cells, such as fluid shear stress and mechanical deformation. These devices have proven valuable for evaluating the effectiveness of anticancer drugs.[112 113]

1.7 Hydrogel 3D Models

The hydrogels have emerged as particularly suitable biomaterials for developing an in vitro model owing to their similarity in features to the extracellular microenvironment of the native ECM.[114] Some of the in vitro models mentioned in previous point can use hydrogel as matrix. As previously said, the behaviour of the cells is influenced by the surrounding matrix. Therefore, when selecting a substrate, diverse substrate properties, as mechanisms, stiffness, topography, degradation, mechanical loading, and cell adhesive indicators, should be

customised for specific diseases and tissue.[115] Both natural, synthetic and hybrid hydrogels are used in vitro models. They can be crosslinked through chemical or physical mechanisms.[114] There are diverse 3D models that use hydrogel as cell culture platform to mimic the microenvironment of the tissue or disease.[116] They can be used to study cell behaviour, disease progression, drug response, and tissue-specific spread, offering a promising avenue for cancer research and therapeutic development.[117] [118]

1.7.1 Hydrogel as artificial tumour microenvironment

The role of pathological molecular and cellular components within the ECM is clear in the development of tumours. The TME not only possesses distinct characteristics from healthy tissue but also undergoes changes as the disease progresses. Recognizing these aspects implies that cancer can be seen as a disorder of architecture, where spatial interactions between tumour and its surroundings influence disease. These biochemical, mechanical, and stromal cell signals help drive the progression of cancer to metastatic disease.[119] This interaction prompts changes in the cancer cells characteristic and functions, including migration, proliferation, secretion of biological molecules and differentiation.[116] Addressing this problem requires the development of in vitro systems that replicate the TME.[119]

The material chosen to replicate the ECM must permit certain mechanisms to be taken as a good 3D culture medium. In the nature, the ECM is composed of different components distributed heterogeneously, resulting in specific niche microenvironments with specific mechanical (strength, elasticity, and mechanical resistance), physical (elasticity, stiffness, porosity, static architecture, and dynamic deformations of the matrix) and biochemical (mediate or regulate stem cell fate, cell proliferation, cell differentiation, cell migration, and tissue regeneration) properties.[120] [121] These play a large role in regulating and mediating cell behaviour. Cell-matrix adhesion sites establish the communication between cells and the ECM by facilitating physical connections of cellular integrins and cadherins to ligands in the ECM.[122] These focal contacts play a crucial role in cellular processes like migration and mechanotransduction, that necessitate physical interaction with the ECM.[123] Moreover, the ECM engages in mechanical communication with cells, mediated by mechanotransduction proteins that regulate how cells respond to mechanical forces exerted by the ECM, resulting in intracellular tensile responses. Cells can perceive various mechanical stimuli, including shear stress, membrane tension, force, strain, stiffness, and drag force.[124] Through the mechanotransduction systems, the diverse mechanical cues from the ECM are transformed into biochemical signals that trigger signalling cascades regulating processes such as transcription, proliferation, migration, and more cellular activities. Notably, ECM biomechanics

are not only critical for cellular functions but also orchestrate processes at the tissue and organ levels, including tissue differentiation, morphogenesis, and overall development.[125]

Hydrogels consist of water-absorbent polymeric networks, capable of retaining large amounts of water. This hydration and porosity, resemble the natural 3D tissue structure, and offer improved exchange of nutrients, gases among the cells and removal of waste products.[126] These polymers have the advantage of being capable of adjust their chemical, physical and biological properties, including stiffness, topography and degradation to replicate the natural tissue, being ideal candidates for use as 3D platforms.[116] [127] There are considerations to take into account when define the hydrogel to be used as matrices.

The cell adhesion is a pivotal process that facilitates the binding of cells to both ECM components and neighbouring cells. To achieve the proper support model some adhesion motifs are added. They offer precise control over cell behaviour. Activations of specific signalling pathways are imperative for driving cell growth, differentiation, and viability. So, specific biochemical cues, such as growth factors, must be incorporated into hydrogels for underscores their role in orchestrating signalling pathways just like it happens in the TME.[128]

Regarding the degradation and remodelling of the matrix, motifs sensitive to MPP degradation can be incorporated into the hydrogel systems to facilitate cell spreading and self-assembly. Incorporating labile bonds or photolabile groups enables controlled degradation rates. Altering hydrogel molecular weight affects physical properties. The goal is to replicate the natural extracellular matrix's biochemical properties, enabling defined hydrogels to support organoid growth, expansion, morphogenesis, and physiological functionalities. Hydrogel mechanical properties like viscoelasticity, stiffness, and stress relaxation can be adjusted by changing prepolymer factors such as molecular weight, structure, and crosslinking density. This customization enables tailored matrices for specific organoid cultures. Hydrogel stiffness can be increased by adjusting molecular weight, crosslinking density, and supramolecular interactions to suit different organoid types.[128] [129]

3D in vitro models replicate vascular networks within tumour environments by using hydrogels and multicellular systems to mimic endothelial cell growth affiliated to blood vessels. Biomaterials imitating the extracellular matrix and chemical signals promoting vessel development are integrated, while microfluidic technology simulates blood flow. These models provide insights into interactions between tumour and endothelial cells, angiogenesis, and metastasis, enhancing understanding of cancer biology and therapeutic development. Furthermore, 3D in vitro models can emulate the immune system by incorporating immune cells (T cells, dendritic cells, macrophages) alongside cancer cells. They simulate immune

signalling and microenvironments to study immune-tumour interactions, immunosuppression, and evasion mechanisms. However, replicating interactions between cancer and immune cells in vitro poses significant challenges, as immune cells, acting as intrinsic environmental sensors, display variable characteristics and behaviours influenced by biochemical and physical cues.[130] [131] The chemical properties are influenced by composition and ligands, significantly impact cellular behaviour. The choice of an appropriate type of hydrogel is needed.

1.7.2 Types of hydrogels

Natural hydrogels are derived from biocompatible and biodegradable polymers found in nature, such as hyaluronic acid and collagen. They offer a safe and supportive environment for cells, promoting growth and differentiation, but may have some variability from batch to batch. The weak mechanical properties and instability limit long-term cell growth. These issues can be overcome by synthetically modifying the polymers. They can be divided into polysaccharide, protein, and peptide-based hydrogels. Polysaccharides, derived from various sources, are suitable for hydrogel development due to their stability, biodegradability, and availability. Alginate, hyaluronic acid, and chitosan-based hydrogels are commonly used in 3D in vitro models.[114]

Alternatively, synthetic hydrogels such as polyethylene glycol and polylactic acid are biologically inert and can be easily customised to meet specific requirements. They serve as a blank slate, permitting but not promoting cellular activity, making them ideal for applications like drug delivery.[132] Covalent bonding of cell adhesion motifs, growth factors, or bioactive molecules to synthetic polymers guides cell growth and organisation directionally. The advantage lies in defining mechanical conditions, such as stiffness, elasticity, and durability and no immunological concerns.[133]

Hybrid hydrogels combine the advantages of both natural and synthetic hydrogels, offering controlled mechanical properties while incorporating the bioactivity of natural polymers. These versatile hydrogel systems are formed by blending different polymers or phases through physical interactions or chemical polymerization.[134] They utilise bioactive molecules from the ECM like growth factors and enzymes, enhancing cell behaviour, growth, and differentiation. Nanomaterials such as silver nanoparticles, carbon nanotubes, graphene oxide, and polymer nanofillers are utilised to improve properties and functionality. These hydrogels are essential in tissue engineering due to their diverse applications, and they ensure proper cell organisation and interactions.[135]

Decellularization is a method used to create ECM-mimetic hydrogel by removing cells and potential immune-triggering components from native tissues while preserving the strong

physical and chemical interactions in the ECM. This process involves various stimuli like chemical, enzymatic, and physical methods, or their combinations. Decellularization retains the internal structure, chemistry, and crosslinking of the native ECM, making it suitable for tissue regeneration without requiring additional crosslinking steps. Non-human tissue sources are also feasible due to washing procedures that eliminate immunogenic elements. However, challenges in sourcing tissues, extensive purification processes, and the potential alteration of ECM components during processing present limitations to this approach. These drawbacks can be overcome by using alternative scaffold materials.[127]

1.7.3 Functionalized Hydrogels

Chemical crosslinking is an extremely versatile technique for enhancing the mechanical characteristics of hydrogels. This process involves modifying the chemical structure of the polymer. Whether utilising synthetic or natural polymers, homopolymers or copolymers, these materials can form three-dimensional networks through molecular entanglements or chemical crosslinking. The introduction of crosslinks between polymer chains has a significant impact on the hydrogel's physical attributes, determined by factors such as the degree of crosslinking and the presence of crystallinity. This alteration can influence properties like elasticity, viscosity, solubility, strength, toughness, melting point, and temperature sensitivity.[136] Physical interactions, such as hydrogen bonds, electrostatic forces, coordination bonds, and hydrophobic interactions, are harnessed to create hydrogels, often adjusting conditions to trigger a transition from a solution to a gel state with responsive properties. Nonetheless, these physical hydrogels exhibit limitations in mechanical strength and plastic flow. Conversely, hydrogels formed through covalent bonding of polymers via chemically active motifs employ techniques like carbodiimide chemistry, aldehyde complementation, radical polymerization, high-energy irradiation, enzyme-enabled biochemistry, and click chemistry. These methods enhance matrix stabilisation, offering greater control and flexibility in hydrogel formation compared to physical mechanisms. Enzymatic crosslinking, particularly with enzymes like peroxidases, transglutaminases, and tyrosinases, has gained traction due to its simplicity and mild reaction conditions, circumventing potential cytotoxic effects linked with chemical crosslinkers. Additionally, electron irradiation has emerged for efficient and precise crosslinking without cytotoxicity, making it suitable for patterned hydrogels in tissue engineering and regenerative medicine, albeit limited by advanced equipment requirements. These chemical crosslinking mechanisms imparts stability but can limit extensibility and toughness. Physical methods like thermal and ionic gelation, self-assembly, and electrostatic interactions offer facile hydrogel production, but their characteristics heavily rely on the inherent properties of the polymers,

affording limited fine-tuning. In contrast, chemical crosslinking methods provide better control over crosslinking, albeit often necessitating polymer modifications that could influence their biofunctionality. The hybrid combination of physical and chemical crosslinking yields distinctive properties. Chemical modifications enable tailored hydrogel functions, such as sustained drug release or growth factor incorporation. Conjugating proteins with crosslinked hybrid networks achieve protein-functionalized immobilised platforms. [137] [138] [139]

1.7.4 Hydrogels production/engineering techniques

Various hydrogel-based 3D culture is frequently utilised, such as bulk hydrogels, porous scaffolds, fibrous scaffolds, microspheres, hydrogel sandwich systems, microwells, and 3D bioprinted constructs (Figure 6). [116] The choice of platform, cell spheroids, aggregates or dispersions is based on the tissue type.

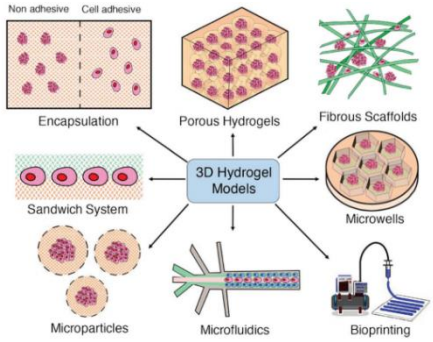

Figure 6 - Schematic illustration of the 3D hydrogel models, including bulk hydrogels (encapsulation), porous hydrogels, fibrous hydrogel scaffolds, hydrogel sandwich systems, hydrogel microparticles, microfluid, and bioprinted scaffolds.[116]

Encapsulation

Cellular encapsulation within 3D bulk hydrogels is a widely utilised technique for advancing tissue construct development. It is the most straightforward, approachable, and the most successful methods in the development of in vitro tissue constructs and clinical transplantation. A variety of synthetic and natural polymers, including PEG, gelatine, and HA, can be employed for cellular encapsulation, with the ability to be crosslinked under conditions favourable to cells. These materials need to be biocompatible and customizable to integrate molecules guiding cell behaviour and differentiation, while also replicating tissue morphology. This technique involves suspending isolated cells within bulk hydrogels and crosslinking them under favourable conditions.[116]

Porous scaffolds

Porous scaffolds are commonly prepared through lyophilization, porogen leaching, and gas foaming, typically yielding pore sizes larger than 100μm. However, these scaffold pores are still considerably bigger than cell sizes (10–20μm), leading to cell-surface interactions resembling those 2D substrates. Cells are directly introduced onto the hydrogel scaffold, with or without gentle agitation. In contrast, inverted colloidal crystals offer remarkable pore uniformity and consistent 3D connectivity, resembling inverse replicas of ordered colloidal sphere arrays. They are crafted using polymeric beads (such as polystyrene and poly(methyl methacrylate), assembled into ordered structures using methods like gravity sedimentation, centrifugation, and more. These organised particles are transformed into a solid construct through annealing., followed by infiltration with a prepolymer solution and crosslinking. After dissolving the spheres with an organic solvent, a hydrogel scaffold with an inverted crystal structure remains. Cell seeding into inverted colloidal crystal constructs employs static, centrifugation, or capillary-force-based techniques. Porous scaffolds primarily facilitate cell clustering to form spheroids and enhance nutrient and oxygen transport within scaffold, though reduced pore size may hinder cellular infiltration rates and even distribution.[116]

Fibrous scaffolds

Fibrous scaffolds are composed of nanoscale (10–300nm) fibres organised into microscale (10–100μm) porous mats, achieved through self-assembly, phase separation, crystallisation, or electrospinning. This nanofibrous structure mimics ECM morphology, promoting cell attachment, proliferation, differentiation, nutrition, and oxygen transport due to its high surface area and interconnectivity. [116 140]

Microencapsulation

Microencapsulation involves confining cells within a hydrogel membrane or droplet, shielding them from the external environment while enhancing cell-cell interactions. Techniques like extrusion, emulsion, and microfluidics are employed to encapsulate cells, with hydrogel materials and methods influencing encapsulation efficiency, size control, and cell density.[116 141]

Sandwich culture technique

The sandwich culture technique involves seeding cells on a 2D hydrogel substrate, allowing attachment. Another hydrogel layer is added atop cells, creating a "sandwich" structure. These double-sandwich systems find application in microfluid platforms or Transwell devices, offering spatial and temporal regulation of various cell types.[116 142]

Microwell technique

The microwell technique involves using a well plate with micrometre-sized compartments where cells gather to form spheroids. These wells can vary in size and shape, affecting cell growth, differentiation, and function. Hydrogel microwell fabrication involves soft lithography where a poly(dimethylsiloxane) (PDMS) stamp is pressed into a hydrogel film, resulting in crosslinked hydrogel-based microwells. The bottom of these microwells can be coated with cell-adhesive proteins for better cell-matrix adhesion. This technique can also be combined with 3D bioprinting for precise spatial control of spheroid location.[116]

3D bioprinting

Bioprinting consists of the deposition of a biomaterial in a predetermined spatial manner, adopting the same approach as a classic 3D printer. Different bioprinting techniques include stereolithography, inkjet-based, laser-assisted, and extrusion-based methods. Stereolithography employs UV light to crosslink photosensitive materials, allowing high-resolution printing with minimal shear stress on cells. Viscosity ranges are broader, and multimaterial integration is possible. Inkjet-based bioprinting dispenses bioink using thermal or acoustic forces, offering high-throughput capabilities and cell survival rates exceeding 90%. Laser-assisted bioprinting uses a laser beam to generate bubbles for bioink ejection, maintaining high cell viability and throughput. Extrusion-based bioprinting deposits continuous filament layers onto a print bed, accommodating various viscosities and offering versatility for creating gradient structures.[143]

Microfluid

Hydrogels are often integrated into Transwell devices or microfluidic devices. Transwell devices consist of permeable membranes separating upper and lower chambers, enabling the study of cellular responses between different cell types and microenvironments. Cells can be arranged in spheroid or sandwich configurations within these chambers. These microfluidic platforms create 3D models with distinct compartments for different cell types connected by microchannels, which prevent cell mixing while allowing soluble bioactive molecules to pass through. It maintains a continuous flow of fluids that enhances nutrients and oxygen transport. These lead to better cell metabolism and function compared to static cultures.[116 144]

Organ on chip

Organ-on-chips are engineered or natural microsystems that replicate tissue functions, like cancer metastasis or inflammation, using human cells or organoids on microchips connected by fluidic microchannels that mimic blood circulation or airflow, emulating human physiological processes. This approach creates 3D models of human tumours and offers better

control over biochemical and biophysical cues, enabling accurate step-by-step of the metastatic progress. To create 3D culture settings, hydrogel-based bioinks are printed into unsealed devices, they can be integrated as a vertical substrate where the cells are seeded onto or in a cell-laden form. This overcomes limitations of traditional cell culture by incorporating dynamic factors and enabling researchers to mimic human physiological features on relevant scales.[145 146]

1.7.5 Applications, advantages, and limitations of hydrogels

The applications of hydrogels are varied. They can be used in a clinical approach, like tissue engineering, as potential solutions for tissue or organ transplantations, injectable hydrogels for drug delivery, tissue regeneration and even contact lenses. In the research field they are used as scaffolds in 3D cultures. These 3D platforms offer a more accurate representation of cell-environment and cell-cell interactions, encompassing biochemically and mechanically, including morphology, cell and environmental stiffness, motility, and signalling. Consequently, they are use in disease studies and drug testing.[147 148 149]

Compared to 2D models, 3D in vitro cancer models provide a truer representation of real tumours, showing enhanced cell viability and essential characteristics like cell-cell and cell-ECM interactions. Hydrogel matrices provide advantages by simulating the microenvironment where these interactions occur, enabling growth factor integration, uniform spheroid distribution, and modelling intricate structures like vessels. Despite these benefits, challenges remain, including the slower process of 3D formation due to cell handling and media changes, as well as initial weak mechanical properties before cross-linking. The complexity of tissue microenvironments and organ structures poses significant challenges in the development of in vitro models. Due to the increased complexity of the structure, the creation of these models requires more time, as does the duration of cell culture. Additionally, stability can be compromised over time, and more stiff hydrogels can impede the diffusion of oxygen and nutrients. However, despite these challenges, 3D models offer a more faithful representation of actual cellular behavior.[107 150 131]

1.7.6 Hydrogel-based models in Prostate Cancer

Hydrogel-based 3D models have emerged as tools for studying the disease and the complex interactions within the surrounding microenvironment. For instance, Xu *et al.* developed a bilayer hydrogel system that effectively mimicked native carcinoma, providing a conducive environment for the growth and arrangement of LNCaP cells. These cells were cultured under biomimetic conditions, enabling effective communication between tumour and

stroma via growth. This system serves as an in vitro platform for drug testing and studying cellular responses to growth factors, offering valuable insights into early-stage cancer progression and facilitating the assessment of innovative anticancer therapies targeting metastasis.[151] An model that mimics early-stage cancer progression, making it valuable for testing innovative anticancer therapies targeting metastasis, was addressed by Pol M. *et al.*, who created a biomimetic hydrogel platform that's enzymatically degradable and dynamically adjustable, allows the incorporation of peptides, reflecting tumour growth and metastasis.[152] Bidarra S. *et al*. addressed the interconversion between epithelial and mesenchymal states, a crucial mechanism in metastatic cancer, like PCa. They employed soft alginate hydrogels functionalized with cell-adhesion peptides to create a 3D matrix that mimics the microenvironment.[153] These few studies demonstrate that hydrogel-based 3D models hold promise as effective tools for drug testing and enhancing our understanding of disease processes.

2. Methodology

The primary focus of this monograph was investigating 3D techniques that can mimic the microenvironment of prostate cancer, and the research for this paper was conducted from January 2023 to August 2023. The approach employed was qualitative, involving the identification of relevant keywords such as "prostate cancer", "3D models" and "cancer environment". A preliminary screening of available articles was conducted using PubMed, Google Scholar, and the Web of Science. To obtain narrowly defined and relevant articles, the "Advanced Search" tool and Boolean operators were used to combine these terms. The selection of data followed a specific criterion, which involved checking whether the title contained the key terms, followed by a meticulous analysis of the abstract, where the relevance was determined. When aligned with the objectives of this paper, the journals and their impact factors on the articles were analysed. Furthermore, to obtain more knowledge, the articles cited on the reference list of previously selected articles were evaluated. To verify the accuracy of the information, a comparison was made between the older articles and the more recent ones.

Mendeley was used to reunite the eligible articles and cite them.

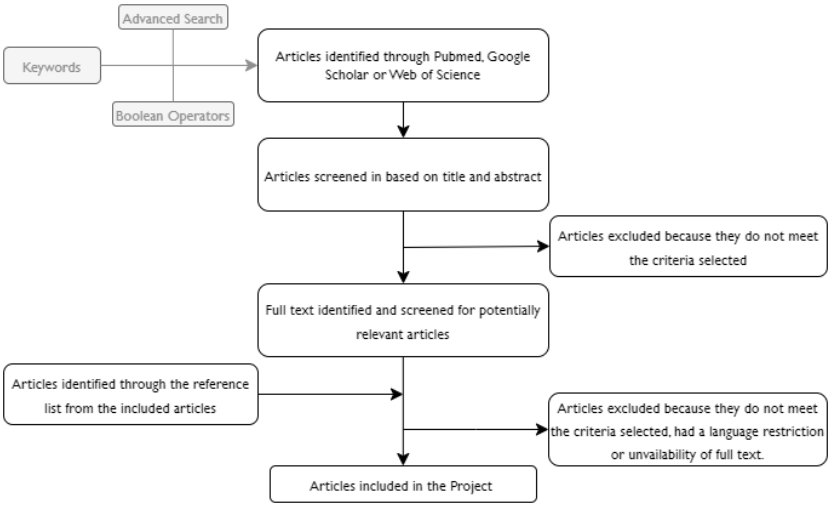

Figure 7 - Flowchart of the methodology.

3. Critical Analysis

PCa, the second most common cancer affecting men worldwide, exhibits heterogeneous behaviour due to its intratumoral variability and associated cellular plasticity. Its epithelium consists of three cell types: luminal, basal, and neuroendocrine. Their differentiation during organogenesis with their differentiation reliant on the stroma, and vice versa. Androgens orchestrate these processes, acting in the UGM to trigger epithelial development and in the UGE to induce stromal differentiation. This interdependent relationship persists throughout a man's life, pivotal for prostate maturation and homeostasis. Epithelial cells remain active postnatally, where unipotent and bipotent cells differentiate. After adolescence and the completion of maturation, unipotent cells maintain homeostasis with reduced activity. Androgen regulation occurs through the hypothalamic–pituitary–gonadal axis, with the majority produced in the prostate's Leydig cells in response to other hormonal stimuli. The stroma facilitates epithelial growth through andromedins' stimulation. These compartments harbour crucial stem cells for their maintenance.

Prostate cancer follows a stepwise progression, starting epithelial structural alterations, luminal cell numbers increase while basal cells decrease. Subsequently, localised cancer emerges, followed by tissue invasion, culminating in distant organ metastasis. Cancer cells of origin can arise from either luminal or basal cells. There are two premises for cell phenotype change. One argues that the tumour arises from a series of oncogenic changes, while the other suggests it originates from the differentiation of a cancer stem cell or dedifferentiation of an adult cell into a cancer stem cell. The luminal phenotype suggests that the originating cell belongs to this category. These cancer stem cells play an essential role in both metastasis and therapy resistance.

Metastasis arises from cells' remarkable plasticity, necessitating an epithelial-mesenchymal transition. Epithelial cells adopt a mesenchymal-like state, gaining stem cell traits. Cells maintaining plasticity during this transition are prone to instigate metastases. Stromal components profoundly influence metastasis, as inflammatory cells facilitate epithelial-mesenchymal transition, enabling detachment and intravasation of circulating tumour cells. Mediators like CAFs and myeloid progenitor cells guide circulating tumour cells, shielded by platelets, to specific sites. Exosomes mediate communication between primary and secondary sites within the microenvironment.

The tumour microenvironment results from epithelial-stromal interactions, supporting cancer cells' survival by inducing stromal changes. Fibrosis replaces smooth muscle cells with cancer-associated fibroblasts, new blood and lymphatic vessels form, and inflammatory cells

infiltrate the area. Hypoxia and increased tumour-associated macrophages occur, modifying the extracellular matrix, leading to a stiffer matrix. These alterations create a reactive stroma that fuels cancer cell proliferation, aggression, and treatment response. The tumour microenvironment varies among patients and even within the same patient.

Accurate diagnosis guides appropriate therapy. Diverse physical and biochemical detection methods have improved over time for enhanced accuracy and reduced limitations. Evaluating PSA concentration in the blood, secreted by luminal prostate cells, is a widely used method due to its simplicity. Suspicion warrants biopsies for tissue histology assessment. The Gleason system scores tissue differentiation and aggressiveness. Therapy selection considers three levels of prostate cancer: localised, locally advanced, and metastatic. Hormonal therapy, involving physical or chemical castration, is the gold standard for metastatic cases. While it offers benefits like tumour regression and improved overall survival, resistance eventually develops. Resistance mechanisms include a variety of factors, such as AR amplification, hypersensitivity, mutations, androgen-independent AR activation, alternative androgen production, AR variants, and progression to neuroendocrine cancer. The tumour microenvironment greatly affects these resistance processes.

Therapeutic approaches must be adjusted accordingly, utilising chemotherapy and immunotherapy. However, resistance arising from the influence of the tumour microenvironment is observed. Experimental models are essential for advancing prostate cancer knowledge. In vitro models are widely used, typically involving two-dimensional cell cultures. However, these fail to replicate key aspects of cancer occurrence, progression, and maintenance, such as vascularization, the tumour microenvironment, cellular interactions, and the extracellular matrix. To overcome these limitations, three-dimensional models have been developed.

Hydrogel-based models are prominent due to their ability to mimic the tumour microenvironment, which influences therapy resistance, metastasis, and progression to aggressive cancer. This mimicry is achieved through their structure resembling natural tissues, integration of biological signals, growth factors, immune cells, vascularization mimicry, and the ability to choose from a variety of hydrogels that match the extracellular matrix and customise them to suit the microenvironment.

Various techniques can create 3D hydrogel models. Culture techniques have evolved to become increasingly reproducible, with dynamic cultures like organ-on-a-chip being capable of incorporating multiple techniques. These dynamic models can mimic blood circulation and incorporate different microenvironments.

4. Summary

The utilisation of hydrogels can effectively mimic the tumour microenvironment. This is supported by their 3D structure and the ability to tailor their physical, mechanical, and biochemical characteristics. The selection of hydrogel source and its customization through various approaches grants adaptability. Consequently, achieving comparable rigidity, elasticity, and strength, as well as the desired porosity, stable structure, and dynamic matrix deformation becomes feasible. By amalgamating these attributes to closely mirror natural tissue, essential features of the tumour microenvironment are integrated. Introducing immune cells allows for immune system emulation, and the recreation of vascular networks is also attainable. The addition of biochemical cues orchestrates specific signalling pathways. The viability, stability, and growth of prostate cancer cell lines within hydrogel matrices have been demonstrated. The manipulation of hydrogels and incorporation of these mentioned features result in a replicated tumour microenvironment that closely resembles the natural setting. This in vitro mimicry captures processes such as proliferation, differentiation, migration, and the adaptability associated with cancer cells.

Disease progression and therapy resistance are linked to both mechanical signals from the stroma and chemical triggers from cells within the tumour microenvironment. Given that metastasis and resistance pose significant challenges in PCa, the in vitro emulation of these mechanisms provides enhanced insights, the discovery of new therapeutic targets, and a more reproducible platform for therapy assessment.

5. Conclusions

PCa is a cancer that affects many men around the world. In its advanced state, it presents a limited therapeutic choice and over time ends up developing resistance, both to conventional therapy and immunotherapy. This gland has a strong dependence on the stroma, since the organogenesis to glandular homeostasis, the interactions between epithelium and stroma exist. When malignancy occurs, the complex and heterogeneous microenvironment surrounding the tumour plays a crucial role in the inherent plasticity of this tumour, contributing to disease progression and resistance to therapy. Therefore, the use of in vitro 3D models to study this cancer is a need. There are different types of supports for cell cultures, but the hydrogels stand out. This is because the identical structure, the possibility of personalising their different properties, enable cell-cell and cell-ECM interactions, add biochemical cues, and maintain higher cell viability. This allows the recreation of key characteristics of this tumour

microenvironment, enabling researchers to access potential therapies and explore tumour mechanisms within a more related and representative context.

It is important to note that beside all the advantages, limitations exist. The hydrogel continues to be less complex than the natural tissue, and the heterogeneity of the disease has considerable importance. Since the microenvironment changes between tissue and in the tissue because of certain alterations as cancer, they cannot be standardised, and the costs increase.

Over the years, the hydrogel-based 3D models have improved to address the limitations associated with their predecessors. The use of more dynamic hydrogels is crucial for a closer approximation to reality.

By utilising the suitable model, a deeper understanding of the disease can be attained, leading to improved therapy efficacy, enhanced patient quality of life, and more accurate prognostic outcomes.

References

1. SUNG, Hyuna *et al.* - Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. **CA: A Cancer Journal for Clinicians**. ISSN 0007-9235. 71:3 (2021) 209–249. doi: 10.3322/CAAC.21660.

2. SIEGEL MPH, Rebecca L. *et al.* - Cancer statistics, 2023. 2023). doi: 10.3322/caac.21763.

3. RAWLA, Prashanth - Epidemiology of Prostate Cancer. **World Journal of Oncology**. ISSN 1920-4531. 10:2 (2019) 63–89. doi: 10.14740/wjon1191.

4. VOIGT, Jeffrey D. *et al.* - Use of the 4Kscore Test to Predict the Risk of Aggressive Prostate Cancer Prior to Prostate Biopsy: Overall Cost Savings and Improved Quality of Care to the US Healthcare System. **Reviews in Urology •**. 19:1 (2017). doi: 10.3909/riu0753.

5. TIWARI, Ritika; MANZAR, Nishat; ATEEQ, Bushra - Dynamics of Cellular Plasticity in Prostate Cancer Progression. **Frontiers in Molecular Biosciences**. ISSN 2296889X. 7:2020). doi: 10.3389/fmolb.2020.00130.

6. ZHANG, Weiyu *et al.* - Global Burden of Prostate Cancer and Association with Socioeconomic Status, 1990–2019: A Systematic Analysis from the Global Burden of Disease Study. [s.d.]). doi: 10.1007/s44197-023-00103-6.

7. TOIVANEN, Roxanne; SHEN, Michael M. - Prostate organogenesis: tissue induction, hormonal regulation and cell type specification. 2017). doi: 10.1242/dev.148270.

8. TIMMS, Barry G. - c. **Differentiation**. ISSN 14320436. 76:6 (2008) 565–577. doi: 10.1111/J.1432-0436.2008.00278.X.

9. ALVES, Edilaine F. *et al.* - Histological and quantitative analyzes of the stromal and acinar components of normal human prostate zones. 2018). doi: 10.1002/pros.23472.

10. ITTMANN, Michael - Anatomy and histology of the human and murine prostate. **Cold Spring Harbor Perspectives in Medicine**. ISSN 21571422. 8:5 (2018). doi: 10.1101/cshperspect.a030346.

11. FINE, Samson W.; REUTER, Victor E. - Anatomy of the prostate revisited: implications for prostate biopsy and zonal origins of prostate cancer. [s.d.]). doi: 10.1111/j.1365-2559.2011.04004.x.

12. AMORY, John K.; BREMNER, William J. - Regulation of testicular function in men: implications for male hormonal contraceptive development. **Journal of Steroid**

Biochemistry & Molecular Biology. 85:2003) 357–361. doi: 10.1016/S0960-0760(03) 00205-X.

13. ANAWALT, Bradley D.; BRAUNSTEIN, Glenn D. - 2. Testes. [s.d.]).

14. FRANCIS, Jeffrey C.; SWAIN, Amanda - Prostate Organogenesis. 2018). doi: 10.1101 /cshperspect. 030353.

15. CUNHA, Gerald R. *et al.* - Hormonal, cellular, and molecular regulation of normal and neoplastic prostatic development. **Journal of Steroid Biochemistry & Molecular Biology**. 92:2004) 221–236. doi: 10.1016/j.jsbmb.2004.10.017.

16. PRINS, Gail S.; KORACH, Kenneth S. - The Role of Estrogens and Estrogen Receptors in Normal Prostate Growth and Disease. **Steroids**. ISSN 0039128X. 73:3 (2008) 233. doi: 10.1016/J.STEROIDS.2007.10.013.

17. OUSSET, Marielle *et al.* - Multipotent and unipotent progenitors contribute to prostate postnatal development. **Nature Cell Biology**. ISSN 1465-7392. 14:11 (2012) 1131–1138. doi: 10.1038/ncb2600.

18. KARTHAUS, Wouter R. *et al.* - Identification of multipotent luminal progenitor cells in human prostate organoid cultures. **Cell**. ISSN 10974172. 159:1 (2014) 163–175. doi: 10.1016/j.cell.2014.08.017.

19. WANG, Zhu A. *et al.* - Lineage analysis of basal epithelial cells reveals their unexpected plasticity and supports a cell-of-origin model for prostate cancer heterogeneity. **Nature Cell Biology**. ISSN 14657392. 15:3 (2013) 274–283. doi: 10.1038/ncb2697.

20. GANGAVARAPU, Kalyan J. *et al.* - Role of prostate stem cells and treatment strategies in benign prostate hyperplasia. **American Journal of Clinical and Experimental Urology**. ISSN 2330-1910. 10:3 (2022) 154.

21. HÄGGLÖF, Christina; BERGH, Anders - The Stroma—A Key Regulator in Prostate Function and Malignancy. **Cancers**. ISSN 20726694. 4:2 (2012) 531. doi: 10.3390/CANCER S4020531.

22. SHEN, Michael M.; ABATE-SHEN, Cory - Molecular genetics of prostate cancer: new prospects for old challenges. [s.d.]). doi: 10.1101/gad.1965810.

23. WANG, Guocan *et al.* - Genetics and biology of prostate cancer. 2018). doi: 10.110/gad. 315739.

24. HUMPHREY, Peter A. - Histopathology of Prostate Cancer. [s.d.]). doi: 10.1101/csh perspect.a030411.

25. BRAWER, Michael K. - SELECTIVE ESTROGEN RECEPTOR MODULATORS Prostatic Intraepithelial Neoplasia: An Overview. **REVIEWS IN UROLOGY**. 7:2005).

26. JONES, D. Holstead *et al.* - Regulation of cancer cell migration and bone metastasis by RANKL. **Nature**. ISSN 1476-4687. 440:7084 (2006) 692–696. doi: 10.1038/NATURE04524.

27. SHEN, Michael M.; ABATE-SHEN, Cory - Molecular genetics of prostate cancer: new prospects for old challenges. [s.d.]). doi: 10.1101/gad.1965810.

28. BAHMAD, Hisham F. *et al.* - Tumor Microenvironment in Prostate Cancer: Toward Identification of Novel Molecular Biomarkers for Diagnosis, Prognosis, and Therapy Development. **Frontiers in Genetics**. ISSN 16648021. 12:2021) 652747. doi: 10.3389 /FGENE.2021.652747/BIBTEX.

29. HUANG, Tianzhi *et al.* - Stem cell programs in cancer initiation, progression, and therapy resistance. **Theranostics**. ISSN 18387640. 10:19 (2020) 8721. doi: 10.7150/THNO. 41648.

30. DONDOSSOLA, Eleonora; PAINDELLI, Claudia - In vitro three-dimensional modeling for prostate cancer. Em **Biomaterials for 3D Tumor Modeling**. [S.l.] : Elsevier, 2020. ISBN 9780128181287. p. 251–286.

31. AW YONG, Koh Meng *et al.* - Mechanotransduction-Induced Reversible Phenotypic Switching in Prostate Cancer Cells. **Biophysical Journal**. ISSN 0006-3495. 112:6 (2017) 1236–1245. doi: 10.1016/J.BPJ.2017.02.012.

32. DATTA, Kaustubh *et al.* - Mechanism of lymph node metastasis in prostate cancer. **Future oncology (London, England)**. ISSN 14796694. 6:5 (2010) 823. doi: 10.2217/FON.10.33.

33. BODY, Jean Jacques; CASIMIRO, Sandra; COSTA, Luís - Targeting bone metastases in prostate cancer: improving clinical outcome. **Nature reviews. Urology**. ISSN 1759-4820. 12:6 (2015) 340–356. doi: 10.1038/NRUROL.2015.90.

34. MITTAL, Vivek - Epithelial Mesenchymal Transition in Tumor Metastasis. **Annual review of pathology**. ISSN 1553-4014. 13:2018) 395–412. doi: 10.1146/ANNUREV-PATHOL-020117-043854.

35. BRABLETZ, Thomas *et al.* - EMT in cancer. **Nature reviews. Cancer**. ISSN 1474-1768. 18:2 (2018) 128–134. doi: 10.1038/NRC.2017.118.

36. ACETO, Nicola *et al.* - En Route to Metastasis: Circulating Tumor Cell Clusters and Epithelial-to-Mesenchymal Transition. **Trends in cancer**. ISSN 2405-8025. 1:1 (2015) 44–52. doi: 10.1016/J.TRECAN.2015.07.006.

37. SOTTNIK, J. L. *et al.* - Tumor-Induced Pressure in the Bone Microenvironment Causes Osteocytes to Promote the Growth of Prostate Cancer Bone Metastases. **Cancer Research**. ISSN 0008-5472. 75:11 (2015) 2151–2158. doi: 10.1158/0008-5472.can-14-2493.

38. QUAIL, Daniela F.; JOYCE, Johanna A. - Microenvironmental regulation of tumor progression and metastasis. **Nature medicine**. ISSN 10788956. 19:11 (2013) 1423. doi: 10.1038/NM.3394.

39. CIEZA-BORRELLA, Clara *et al.* - Tumor Microenvironment in Prostate Cancer: Toward Identification of Novel Molecular Biomarkers for Diagnosis, Prognosis, and Therapy Development. **Front. Genet**. 12:2021) 652747. doi: 10.3389/fgene.2021.652747.

40. HEIDEGGER, Isabel *et al.* - Comprehensive characterization of the prostate tumor microenvironment identifies CXCR4/CXCL12 crosstalk as a novel antiangiogenic therapeutic target in prostate cancer. **Molecular Cancer**. ISSN 14764598. 21:1 (2022) 1–20. doi: 10.1186 /S12943-022-01597-7/FIGURES/8.

41. GENG, Hao *et al.* - Interplay between hypoxia and androgen controls a metabolic switch conferring resistance to androgen/AR-targeted therapy. **Nature Communications 2018 9:1**. ISSN 2041-1723. 9:1 (2018) 1–16. doi: 10.1038/s41467-018-07411-7.

42. RUNA, F. *et al.* - MOLECULAR BIOLOGY OF PROSTATE CANCER (M KRUITHOF-DE JULIO, SECTION EDITOR) Tumor Microenvironment Heterogeneity: Challenges and Opportunities. 2017). doi: 10.1007/s40610-017-0073-7.

43. LITWIN, Mark S.; TAN, Hung Jui - The diagnosis and treatment of prostate cancer: A review. **JAMA - Journal of the American Medical Association**. ISSN 15383598. 317:24 (2017) 2532–2542. doi: 10.1001/jama.2017.7248.

44. MOTTET N *et al.* - EAU-EANM-ESTRO-ESUR-ISUP-SIOG-Guidelines-on-Prostate-Cancer-2023. Em . ISBN 978-94-92671-19-6.

45. HALPERN, Joshua A. *et al.* - Utility of Digital Rectal Examination (DRE) as an Adjunct to Prostate Specific Antigen (PSA) in the Detection of Clinically Significant Prostate Cancer HHS Public Access. **J Urol**. 199:4 (2018) 947–953. doi: 10.1016/j.juro.2017.10.021.

46. PEZARO, C.; WOO, H. H.; DAVIS, I. D. - Prostate cancer: Measuring PSA. **Internal Medicine Journal**. ISSN 14455994. 44:5 (2014) 433–440. doi: 10.1111/imj.12407.

47. MEDIU, Ridvana; RAMA, Ariol; MEDIU, Naim - Screening for prostate cancer: a study on the free and total prostate specific antigen. **Discoveries**. ISSN 23597232. 9:4 (2021) e139. doi: 10.15190/d.2021.18.

48. MIKOLAJCZYK, Stephen D. *et al.* - **Free Prostate-Specific Antigen in Serum is Becoming More Complex**

49. THOMPSON, Ian M. *et al.* - Prevalence of Prostate Cancer among Men with a Prostate-Specific Antigen Level ≤4.0 ng per Milliliter. **https://doi.org/10.1056/NEJMoa031918**. ISSN 0028-4793. 350:22 (2004) 2239–2246. doi: 10.1056/NEJMOA031918.

50. CATALONA, William J. *et al.* - Comparison of Digital Rectal Examination and Serum Prostate Specific Antigen in the Early Detection of Prostate Cancer: Results of a Multicenter Clinical Trial of 6,630 Men. **Journal of Urology**. ISSN 0022-5347. 197:2S (2017). doi: 10.1016/j.juro.2016.10.073.

51. LOEB, Stacy; CATALONA, William J. - Prostate-specific antigen in clinical practice. **Cancer Letters**. ISSN 03043835. 249:1 (2007) 30–39. doi: 10.1016/j.canlet.2006.12.022.

52. SMEENGE, Martijn *et al.* - Role of transrectal ultrasonography (TRUS) in focal therapy of prostate cancer: Report from a Consensus Panel. **BJU International**. ISSN 14644096. 110:7 (2012) 942–948. doi: 10.1111/j.1464-410X.2012.11072.x.

53. SMEENGE, Martijn *et al.* - Role of transrectal ultrasonography (TRUS) in focal therapy of prostate cancer: Report from a Consensus Panel. **BJU International**. ISSN 14644096. 110:7 (2012) 942–948. doi: 10.1111/j.1464-410X.2012.11072.x.

54. CORREAS, Jean Michel *et al.* - Advanced ultrasound in the diagnosis of prostate cancer. **World Journal of Urology**. ISSN 14338726. 39:3 (2021) 661–676. doi: 10.1007/s00345-020-03193-0.

55. SCHLEMMER, Heinz Peter *et al.* - Bildgebung des Prostatakarzinoms. **Deutsches Arzteblatt International**. ISSN 18660452. 118:42 (2021) 713–719. doi: 10.3238/arztebl .m2021.0309.

56. AHMED, Hashim U. *et al.* - Diagnostic accuracy of multi-parametric MRI and TRUS biopsy in prostate cancer (PROMIS): a paired validating confi rmatory study. **www.thelancet.com**. 389:2017). doi: 10.1016/S0140-6736(16)32401-1.

57. LEEK, J. *et al.* - Prostate-specific membrane antigen: evidence for the existence of a second related human gene. **British Journal of Cancer**. ISSN 0007-0920. 72:3 (1995) 583–588. doi: 10.1038/bjc.1995.377.

58. KINOSHITA, Yoshihisa *et al.* - Expression of prostate-specific membrane antigen in normal and malignant human tissues. **World Journal of Surgery**. ISSN 03642313. 30:4 (2006) 628–636. doi: 10.1007/s00268-005-0544-5.

59. FAROLFI, Andrea *et al.* - Current and Emerging Clinical Applications of PSMA PET Diagnostic Imaging for Prostate Cancer. **Journal of Nuclear Medicine**. ISSN 2159662X. 62:5 (2021) 596–604. doi: 10.2967/jnumed.120.257238.

60. GARCÍA GARZÓN, J. R. *et al.* - La PET/TC con 68 Ga-PSMA en el cáncer de próstata. **Revista Española de Medicina Nuclear e Imagen Molecular**. ISSN 2253654X. 37:2 (2018) 130–138. doi: 10.1016/j.remn.2017.07.004.

61. XUE, Jianxin *et al.* - Comparison between transrectal and transperineal prostate biopsy for detection of prostate cancer: a meta-analysis and trial sequential analysis. [s.d.]).

62. HARNDEN, Patricia *et al.* - Review Should the Gleason grading system for prostate cancer be modifi ed to account for high-grade tertiary components? A systematic review and meta-analysis. 2007). doi: 10.1016/S1470-2045(07)70136-5.

63. HRICAK, Hedvig.; SCARDINO, Peter T. - **Prostate cancer**. [S.l.] : Cambridge University Press, 2009. ISBN 9780521887045.

64. LEE, Christine H.; AKIN-OLUGBADE, Oluyemi; KIRSCHENBAUM, Alexander - Overview of Prostate Anatomy, Histology, and Pathology KEYWORDS Prostate Prostatic intraepithelial neoplasia Adenocarcinoma Mouse model Androgen receptor. **Endocrinol Metab Clin N Am**. 40:2011) 565–575. doi: 10.1016/j.ecl.2011.05.012.

65. HUMPHREY, Peter A. - Gleason grading and prognostic factors in carcinoma of the prostate. [s.d.]). doi: 10.1038/modpathol.3800054.

66. ICZKOWSKI, Kenneth A. *et al.* - Cribriform prostate cancer: Morphologic criteria enabling a diagnosis, based on survey of experts. **Annals of Diagnostic Pathology**. 52:2021) 1092–9134. doi: 10.1016/j.anndiagpath.2021.151733.

67. EGEVAD, Lars *et al.* - International Society of Urological Pathology (ISUP) grading of prostate cancer - An ISUP consensus on contemporary grading. **APMIS**. ISSN 09034641. 124:6 (2016) 433–435. doi: 10.1111/apm.12533.

68. BRIERLEY, J. D.; GOSPODAROWICZ, M. K.; WITTEKIND, C. - **TNM Classification of Malignant Tumours**. 8. ed. Oxford : Wiley, 2017

69. HELSEN, Christine *et al.* - Androgen receptor antagonists for prostate cancer therapy. **Endocrine-Related Cancer**. ISSN 14796821. 21:4 (2014). doi: 10.1530/ERC-13-0545.

70. HASHINE, Katsuyoshi *et al.* - Surveillance biopsy and active treatment during active surveillance for low-risk prostate cancer. [s.d.]). doi: 10.1007/s10147-013-0584-z.

71. LOEB, Stacy *et al.* - Platinum Priority-Prostate Cancer Active Surveillance Versus Watchful Waiting for Localized Prostate Cancer: A Model to Inform Decisions. 2017). doi: 10.1016/j.eururo.2017.07.018.

72. SANDHU, Gurdarshan S. *et al.* - Laparoscopic Prostatectomy for Prostate Cancer Continued Role in Urology. **Surgical Oncology Clinics of NA**. 22:2013) 125–141. doi: 10.1016/j.soc.2012.08.001.

73. POPPEL, Hendrik VAN *et al.* - Open and robotic radical prostatectomy. 2018). doi: 10.1016/j.ajur.2018.12.002.

74. PAZ-MANRIQUE, Roberto *et al.* - Radiation therapy after radical surgery in prostate cancer. 17:1565). doi: 10.3332/ecancer.2023.1565.

75. GRECO, Maria Chiara LO *et al.* - Hypofractionated Radiotherapy in Localized, Low–Intermediate-Risk Prostate Cancer: Current and Future Prospectives. **Medicina**. ISSN 1648-9144. 59:6 (2023) 1144. doi: 10.3390/medicina59061144.

76. ZAORSKY, Nicholas G. *et al.* - Evolution of brachytherapy for prostate cancer. **Nature reviews. Urology**. ISSN 17594820. 14:7 (2017) 415. doi: 10.1038/NRUROL.2017.76.

77. PAZ-MANRIQUE, Roberto *et al.* - Radiation therapy after radical surgery in prostate cancer. 17:1565). doi: 10.3332/ecancer.2023.1565.

78. BOLLA, Michel *et al.* - Short androgen suppression and radiation dose escalation for intermediate-and high-risk localized prostate cancer: Results of EORTC trial 22991. **Journal of Clinical Oncology**. ISSN 15277755. 34:15 (2016) 1748–1756. doi: 10.1200/JCO.2015.64.8055.

79. ZAPATERO, Almudena *et al.* - High-dose radiotherapy with short-term or long-term androgen deprivation in localised prostate cancer (DART01/05 GICOR): A randomised, controlled, phase 3 trial. **The Lancet Oncology**. ISSN 14745488. 16:3 (2015) 320–327. doi: 10.1016/S1470-2045(15)70045-8.

80. HAMDY, Freddie C. *et al.* - 10-Year Outcomes after Monitoring, Surgery, or Radiotherapy for Localized Prostate Cancer. **New England Journal of Medicine**. ISSN 0028-4793. 375:15 (2016) 1415–1424. doi: 10.1056/nejmoa1606220.

81. CHEN, Ronald C. *et al.* - Association between choice of radical prostatectomy, external beam radiotherapy, brachytherapy, or active surveillance and patient-reported quality of life

among men with localized prostate cancer. **JAMA - Journal of the American Medical Association**. ISSN 15383598. 317:11 (2017) 1141–1150. doi: 10.1001/jama.2017.1652.

82. VALERIO, Massimo *et al.* - New and Established Technology in Focal Ablation of the Prostate: A Systematic Review. **European Urology**. ISSN 0302-2838. 71:1 (2017) 17–34. doi: 10.1016/J.EURURO.2016.08.044.

83. ATTA, Mohamed Adel *et al.* - Is there still a role for bilateral orchidectomy in androgen-deprivation therapy for metastatic prostate cancer? **Arab Journal of Urology**. 18:2020). doi: 10.1080/2090598X.2019.1690270.

84. SAAD, Fred; FIZAZI, Karim - Androgen Deprivation Therapy and Secondary Hormone Therapy in the Management of Hormone-sensitive and Castration-resistant Prostate Cancer. [s.d.]). doi: 10.1016/j.urology.2015.07.034.

85. PEJČIĆ, Tomislav *et al.* - Mechanisms of Prostate Cancer Cells Survival and Their Therapeutic Targeting. **International Journal of Molecular Sciences**. ISSN 14220067. 24:3 (2023). doi: 10.3390/ijms24032939.

86. SCHWEIZER, Michael T.; ANTONARAKIS, Emmanuel S. - Abiraterone and other novel androgen-directed strategies for the treatment of prostate cancer: A new era of hormonal therapies is born. **Therapeutic Advances in Urology**. ISSN 17562880. 4:4 (2012) 167–178. doi: 10.1177/1756287212452196.

87. BASU, Sujit; TINDALL, Donald J. - Androgen Action in Prostate Cancer. [s.d.]). doi: 10.1007/s12672-010-0044-4.

88. VISAKORPI, Tapio *et al.* - In vivo amplification of the androgen receptor gene and progression of human prostate cancer. **Nature genetics**. ISSN 1061-4036. 9:4 (1995) 401–406. doi: 10.1038/NG0495-401.

89. NI, Li *et al.* - FKBP51 promotes assembly of the Hsp90 chaperone complex and regulates androgen receptor signaling in prostate cancer cells. **Molecular and cellular biology**. ISSN 1098-5549. 30:5 (2010) 1243–1253. doi: 10.1128/MCB.01891-08.

90. XU, Jianming; WU, Ray-Chang; O'MALLEY, Bert W. - Normal and cancer-related functions of the p160 steroid receptor co-activator (SRC) family. 2009). doi: 10.1038/nrc2695.

91. CHANDRASEKAR, Thenappan *et al.* - Mechanisms of resistance in castration-resistant prostate cancer (CRPC). **Translational Andrology and Urology**. ISSN 22234691. 4:3 (2015) 365. doi: 10.3978/J.ISSN.2223-4683.2015.05.02.

92. NADIMINTY, Nagalakshmi *et al.* - Aberrant activation of the androgen receptor by NF-kappaB2/p52 in prostate cancer cells. **Cancer research**. ISSN 1538-7445. 70:8 (2010) 3309–3319. doi: 10.1158/0008-5472.CAN-09-3703.

93. ZHAO, Yu; TINDALL, Donald J.; HUANG, Haojie - Androgen Receptor Signaling in Castration Resistant Prostate Cancer. Em BALAJI, K. C. (Ed.) - **Managing Metastatic Prostate Cancer In Your Urological Oncology Practice** [Em linha]. Cham : Springer International Publishing, 2016 Disponível em WWW:<URL:https://doi.org/10.1007/978-3-319-31341-2_3>. ISBN 978-3-319-31341-2. p. 23–34.

94. CONTEDUCA, Vincenza *et al.* - Clinical features of neuroendocrine prostate cancer. **European Journal of Cancer**. ISSN 18790852. 121:2019) 7–18. doi: 10.1016/j.ejca.2019.08.011.

95. HUANG, Yu Hua; ZHANG, Ya Qun; HUANG, Jiao Ti - Neuroendocrine cells of prostate cancer: biologic functions and molecular mechanisms. **Asian Journal of Andrology**. ISSN 17457262. 21:3 (2019) 291. doi: 10.4103/AJA.AJA_128_18.

96. CHMP - ANEXO I RESUMO DAS CARACTERÍSTICAS DO MEDICAMENTO - Docetaxel. [s.d.]).

97. CHMP - **ANEXO I RESUMO DAS CARACTERÍSTICAS DO MEDICAMENTO - Cabazitaxel**

98. LIN, Canling *et al.* - Advances in bio-immunotherapy for castration-resistant prostate cancer. [s.d.]). doi: 10.1007/s00432-023-05152-9.

99. KANTOFF, Philip W. *et al.* - Sipuleucel-T Immunotherapy for Castration-Resistant Prostate Cancer. **n engl j med**. 363:5 (2010) 411–433. doi: 10.1056/NEJMoa1001294.

100. HANDA, Shivani *et al.* - Immunotherapy in prostate cancer: current state and future perspectives. **Therapeutic advances in urology**. ISSN 1756-2872. 12:2020). doi: 10.1177/1756287220951404.

101. GRAHAM, Melanie L.; PRESCOTT, Mark J. - The multifactorial role of the 3Rs in shifting the harm-benefit analysis in animal models of disease. **European Journal of Pharmacology**. ISSN 18790712. 759:2015) 19. doi: 10.1016/J.EJPHAR.2015.03.040.

102. GRIENSVEN, Martijn VAN *et al.* - In Vitro Tumor Models: Advantages, Disadvantages, Variables, and Selecting the Right Platform. 4:2016). doi: 10.3389/fbioe.2016.00012.

103. HEPBURN, Anastasia C. *et al.* - Engineering Prostate Cancer from Induced Pluripotent Stem Cells-New Opportunities to Develop Preclinical Tools in Prostate and Prostate Cancer

Studies. **International journal of molecular sciences**. ISSN 1422-0067. 21:3 (2020). doi: 10.3390/IJMS21030905.

104. ELLEM, Stuart J.; DE-JUAN-PARDO, Elena M.; RISBRIDGER, Gail P. - In vitro modeling of the prostate cancer microenvironment. **Advanced Drug Delivery Reviews**. ISSN 0169-409X. 79–80:2014) 214–221. doi: 10.1016/J.ADDR.2014.04.008.

105. MANDUCA, Nicoletta *et al.* - 3D cancer models: One step closer to in vitro human studies OPEN ACCESS EDITED BY. **Frontiers in Immunology frontiersin.org Front. Immunol**. 14:2023) 1175503. doi: 10.3389/fimmu.2023.1175503.

106. XU, Xian; FARACH-CARSON, Mary C.; JIA, Xinqiao - Three-Dimensional In Vitro Tumor Models for Cancer Research and Drug Evaluation. **Biotechnology advances**. ISSN 07349750. 32:7 (2014) 1256. doi: 10.1016/J.BIOTECHADV.2014.07.009.

107. AJJARAPU, Suchitra Maheswari; TIWARI, Apoorv; KUMAR, Sundip - Applications and Utility of Three-Dimensional In Vitro Cell Culture for Therapeutics. **Future Pharmacology**. 3:1 (2023) 213–228. doi: 10.3390/futurepharmacol3010015.

108. WEISWALD, Louis-Bastien *et al.* - Spherical Cancer Models in Tumor Biology. **Neoplasia**. 17:2015) 1. doi: 10.1016/j.neo.2014.12.004.

109. GAO, Dong *et al.* - Organoid Cultures Derived from Patients with Advanced Prostate Cancer. **Cell**. ISSN 0092-8674. 159:1 (2014) 176–187. doi: 10.1016/J.CELL.2014.08.016.

110. ELLEM, Stuart J.; DE-JUAN-PARDO, Elena M.; RISBRIDGER, Gail P. - In vitro modeling of the prostate cancer microenvironment. **Advanced Drug Delivery Reviews**. ISSN 0169-409X. 79–80:2014) 214–221. doi: 10.1016/J.ADDR.2014.04.008.

111. ASNAGHI, M. Adelaide *et al.* - A double-chamber rotating bioreactor for the development of tissue-engineered hollow organs: From concept to clinical trial. **Biomaterials**. ISSN 0142-9612. 30:29 (2009) 5260–5269. doi: 10.1016/J.BIOMATERIALS .2009 .07.018.

112. BERSINI, Simone *et al.* - A microfluidic 3D in vitro model for specificity of breast cancer metastasis to bone. **Biomaterials**. ISSN 0142-9612. 35:8 (2014) 2454–2461. doi: 10.1016/J.BIOMATERIALS.2013.11.050.

113. LEUNG, Chak Ming *et al.* - A guide to the organ-on-a-chip. **Nature Reviews Methods Primers 2022 2:1**. ISSN 2662-8449. 2:1 (2022) 1–29. doi: 10.1038/s43586-022-00118-6.

114. GOMEZ-FLORIT, Manuel *et al.* - Natural-Based Hydrogels for Tissue Engineering Applications. **Molecules**. ISSN 14203049. 25:24 (2020). doi: 10.3390/MOLECULES25245858.

115. GUVENDIREN, Murat; BURDICK, Jason A. - Engineering synthetic hydrogel microenvironments to instruct stem cells. **Current Opinion in Biotechnology**. ISSN 0958-1669. 24:5 (2013) 841–846. doi: 10.1016/J.COPBIO.2013.03.009.

116. LIAW, Chya Yan; JI, Shen; GUVENDIREN, Murat - Engineering 3D Hydrogels for Personalized In Vitro Human Tissue Models. **Advanced Healthcare Materials**. ISSN 21922659. 7:4 (2018). doi: 10.1002/adhm.201701165.

117. DUVAL, Kayla *et al.* - Modeling Physiological Events in 2D vs. 3D Cell Culture. **Physiology**. ISSN 15489221. 32:4 (2017) 266. doi: 10.1152/PHYSIOL.00036.2016.

118. PARK, Kyung Min; GERECHT, Sharon - Polymeric hydrogels as artificial extracellular microenvironments for cancer research. **European Polymer Journal**. ISSN 0014-3057. 72:2015) 507–513. doi: 10.1016/J.EURPOLYMJ.2015.06.030.

119. GILL, Bartley J.; WEST, Jennifer L. - Modeling the tumor extracellular matrix: Tissue engineering tools repurposed towards new frontiers in cancer biology. **Journal of biomechanics**. ISSN 1873-2380. 47:9 (2014) 1969–1978. doi: 10.1016/J.JBIOMECH.2013.09.029.

120. YUE, Beatrice - Biology of the extracellular matrix: an overview. **Journal of glaucoma**. ISSN 1536-481X. 23:8 Suppl 1 (2014) S20–S23. doi: 10.1097/IJG.00000000000000108.

121. NELSON, Celeste M.; BISSELL, Mina J. - Of Extracellular Matrix, Scaffolds, and Signaling: Tissue Architecture Regulates Development, Homeostasis, and Cancer. **https://doi.org/10.1146/annurev.cellbio.22.010305.104315**. ISSN 10810706. 22:2006) 287–309. doi: 10.1146/ANNUREV.CELLBIO.22.010305.104315.

122. MUI, Keeley L.; CHEN, Christopher S.; ASSOIAN, Richard K. - The mechanical regulation of integrin-cadherin crosstalk organizes cells, signaling and forces. **Journal of cell science**. ISSN 1477-9137. 129:6 (2016) 1093–1100. doi: 10.1242/JCS.183699.

123. SEONG, Jihye; WANG, Ning; WANG, Yingxiao - Mechanotransduction at focal adhesions: from physiology to cancer development. **Journal of cellular and molecular medicine**. ISSN 1582-4934. 17:5 (2013) 597–604. doi: 10.1111/JCMM.12045.

124. WANG, Ning - Review of cellular mechanotransduction. **Journal of Physics D: Applied Physics**. ISSN 0022-3727. 50:23 (2017) 233002. doi: 10.1088/1361-6463/AA6E18.

125. WOZNIAK, Michele A.; CHEN, Christopher S. - Mechanotransduction in development: a growing role for contractility. **Nature Reviews Molecular Cell Biology 2009 10:1**. ISSN 1471-0080. 10:1 (2009) 34–43. doi: 10.1038/nrm2592.

126. RADEMAKERS, Timo *et al.* - Oxygen and nutrient delivery in tissue engineering: Approaches to graft vascularization. **Journal of Tissue Engineering and Regenerative Medicine**. ISSN 19327005. 13:10 (2019) 1815. doi: 10.1002/TERM.2932.

127. XU, Fei *et al.* - Hydrogels for Tissue Engineering: Addressing Key Design Needs Toward Clinical Translation. **Frontiers in Bioengineering and Biotechnology**. ISSN 22964185. 10:2022). doi: 10.3389/fbioe.2022.849831.

128. GAN, Zhongqiao *et al.* - Recent advances in defined hydrogels in organoid research. **Bioactive Materials**. ISSN 2452-199X. 28:2023) 386–401. doi: 10.1016/J.BIOACTMAT.2023.06.004.

129. ZHAO, Weifeng *et al.* - Functional Hydrogels With Tunable Structures and Properties for Tissue Engineering Applications. 6:2018) 499. doi: 10.3389/fchem.2018.00499.

130. FERNANDO, Kanishka *et al.* - Hydrogels to engineer tumor microenvironments: In vitro. **Biomaterials Science**. ISSN 20474849. 9:7 (2021) 2362–2383. doi: 10.1039/ d0bm01943g.

131. SONG, Hyun Ho Greco; PARK, Kyung Min; GERECHT, Sharon - Hydrogels to Model 3D in vitro Microenvironment of Tumor Vascularization. **Advanced drug delivery reviews**. ISSN 18728294. 0:2014) 19. doi: 10.1016/J.ADDR.2014.06.002.

132. DUPIN, Damien *et al.* - Polymer-Based Hydrogels Applied in Drug Delivery: An Overview. **Gels 2023, Vol. 9, Page 523**. ISSN 2310-2861. 9:7 (2023) 523. doi: 10.3390/ GELS9070523.

133. GUO, Baolin; MA, Peter X. - Synthetic biodegradable functional polymers for tissue engineering: a brief review. **Science China. Chemistry**. ISSN 16747291. 57:4 (2014) 490. doi: 10.1007/S11426-014-5086-Y.

134. LAU, Hang Kuen; KIICK, Kristi L. - Opportunities for Multicomponent Hybrid Hydrogels in Biomedical Applications. **Biomacromolecules**. ISSN 1525-7797. 16:1 (2014) 28–42. doi: 10.1021/BM501361C.

135. CAI, Mao Hua *et al.* - Design and Development of Hybrid Hydrogels for Biomedical Applications: Recent Trends in Anticancer Drug Delivery and Tissue Engineering. **Frontiers**

in Bioengineering and Biotechnology. ISSN 22964185. 9:2021) 630943. doi: 10.3389/FBIOE.2021.630943.

136. MAITRA, Jaya; KUMAR SHUKLA, Vivek - Cross-linking in Hydrogels-A Review. **American Journal of Polymer Science**. 2014:2 (2014) 25–31. doi: 10.5923/j.ajps.20140402.01.

137. GOMEZ-FLORIT, Manuel *et al.* - Natural-Based Hydrogels for Tissue Engineering Applications. **Molecules**. ISSN 14203049. 25:24 (2020). doi: 10.3390/MOLECULES25245858.

138. SAYED, Marwa M. El - Production of Polymer Hydrogel Composites and Their Applications. 31:2023) 2855–2879. doi: 10.1007/s10924-023-02796-z.

139. CALIARI, Steven R.; BURDICK, Jason A. - A practical guide to hydrogels for cell culture. **Nature Methods 2016 13:5**. ISSN 1548-7105. 13:5 (2016) 405–414. doi: 10.1038/nmeth.3839.

140. SMITH, L. A.; MA, P. X. - Nano-fibrous scaffolds for tissue engineering. **Colloids and surfaces. B, Biointerfaces**. ISSN 0927-7765. 39:3 (2004) 125–131. doi: 10.1016/J.COLSURFB.2003.12.004.

141. GASPERINI, Luca; MANO, João F.; REIS, Rui L. - Natural polymers for the microencapsulation of cells. **Journal of the Royal Society, Interface**. ISSN 1742-5662. 11:100 (2014). doi: 10.1098/RSIF.2014.0817.

142. BJØRGE, Isabel M. *et al.* - Cell Behavior within Nanogrooved Sandwich Culture Systems. **Small (Weinheim an der Bergstrasse, Germany)**. ISSN 1613-6829. 16:31 (2020). doi: 10.1002/SMLL.202001975.

143. PARODI, Ilaria *et al.* - 3D Bioprinting as a Powerful Technique for Recreating the Tumor Microenvironment. **Gels (Basel, Switzerland)**. ISSN 2310-2861. 9:6 (2023) 482. doi: 10.3390/GELS9060482.

144. AKTHER, Fahima *et al.* - Hydrogels as artificial matrices for cell seeding in microfluidic devices. **RSC Advances**. ISSN 20462069. 10:71 (2020) 43682–43703. doi: 10.1039/D0RA08566A.

145. LEUNG, Chak Ming *et al.* - A guide to the organ-on-a-chip. **Nature Reviews Methods Primers 2022 2:1**. ISSN 2662-8449. 2:1 (2022) 1–29. doi: 10.1038/s43586-022-00118-6.

146. KUTLUK, Hazal *et al.* - Integration of Extracellular Matrices into Organ-on-Chip Systems. **Advanced Healthcare Materials**. ISSN 2192-2659. 12:20 (2023) 2203256. doi: 10.1002/ADHM.202203256.

147. JUBELIN, Camille *et al.* - Three-dimensional in vitro culture models in oncology research. **Cell & Bioscience 2022 12:1**. ISSN 2045-3701. 12:1 (2022) 1–28. doi: 10.1186/S13578-022-00887-3.

148. NISHIMURA, Yuhei *et al.* - Three-Dimensional in Vitro Cell Culture Models in Drug Discovery and Drug Repositioning. **Front. Pharmacol**. 9:2018) 6. doi: 10.3389/fphar.2018.00006.

149. SAHAN, Ayse Z.; BADAY, Murat; PATEL, Chirag B. - Biomimetic Hydrogels in the Study of Cancer Mechanobiology: Overview, Biomedical Applications, and Future Perspectives. **Gels**. ISSN 23102861. 8:8 (2022). doi: 10.3390/GELS8080496.

150. BARBOSA, Mélanie A. G. *et al.* - 3D Cell Culture Models as Recapitulators of the Tumor Microenvironment for the Screening of Anti-Cancer Drugs. **Cancers**. ISSN 20726694. 14:1 (2022) 190. doi: 10.3390/CANCERS14010190.

151. XU, Xian *et al.* - Recreating the tumor microenvironment in a bilayer, hyaluronic acid hydrogel construct for the growth of prostate cancer spheroids. **Biomaterials**. ISSN 0142-9612. 33:35 (2012) 9049–9060. doi: 10.1016/J.BIOMATERIALS.2012.08.061.

152. POL, Mugdha *et al.* - Dynamic modulation of matrix adhesiveness induces epithelial-to-mesenchymal transition in prostate cancer cells in 3D. **Biomaterials**. ISSN 0142-9612. 299:2023) 122180. doi: 10.1016/J.BIOMATERIALS.2023.122180.

153. BIDARRA, S. J. *et al.* - A 3D in vitro model to explore the inter-conversion between epithelial and mesenchymal states during EMT and its reversion . **Nature Publishing Group**. 2016). doi: 10.1038/srep27072.